Author response related to the manuscript "Oxidative potential of fine particles at urban and rural sites in eastern and western Japan: Effects of transboundary transport from continental Asia and local emissions" by Nishita-Hara et al.

We are grateful to the reviewers for their thoughtful and constructive comments on our manuscript. We have carefully addressed all the points raised and revised the manuscript accordingly. Detailed responses to each comment are provided below. The referees' comments are shown in blue and our responses in black.

Comment 1 from Referee #1

Table 1: Recalculate the mass of the collected sample to the average mass concentration of $PM_{2.5}$ during the sampling periods and compare it with the corresponding data from nearby monitoring stations. Data from monitoring stations provide also as average concentrations. Give all results to 3 valid digits.

Response

We thank the reviewer for this comment. In this study, a cyclone sampler with a cut-off aerodynamic diameter of 0.2 μm was used; therefore, particles smaller than approximately 0.2 μm in diameter were not collected. Moreover, not all particles within the diameter range of 0.2–2.5 μm can be recovered, because complete recovery of particles captured inside the cyclone is not possible, as explained in lines 119-122 in the revised manuscript. Consequently, the collected sample mass does not represent $PM_{2.5}$ mass in the atmosphere. Because of these methodological differences, recalculating the sample mass as $PM_{2.5}$ mass concentration and directly comparing it with $PM_{2.5}$ data reported by nearby monitoring stations would be inappropriate. The purpose of presenting the average $PM_{2.5}$ concentrations in Table 1 is to describe the atmospheric characteristics at the different sampling sites, not to compare them with the collected sample mass.

Following the reviewer's suggestion, the $PM_{2.5}$ mass concentration values from the monitoring stations in Table 1 have been revised accordingly, changing the reported values from median to mean values. To reflect the measurement precision of the $PM_{2.5}$ mass concentration data, the mean values are presented with one decimal place.

Comment 2 from Referee #1

Lines 144-154: The procedure used to measure OP^{DCFH} differs from similar papers; in particular, there is no positive control using zymosan. The authors should discuss the reasons for their choice in detail. The relative percentages used as the unit for OP^{DCFH} measurement then prevent direct comparison with other papers.

Response (1)

We thank the reviewer for this important comment regarding the assay validation and comparability. To address the comment, an additional experiment was conducted to confirm the responsiveness of the CM-H_2DCFDA assay. Instead of using zymosan, we employed a chemically defined ROS-generating system (H_2O_2 and iron ions) as a positive control. A549 cells loaded with CM-H_2DCFDA were exposed to a mixture of H_2O_2 (100 µM) and Fe ions (100 µM or 200 µM). This exposure significantly increased DCF fluorescence compared with the control, confirming that the assay system used in this study responds to intracellular ROS generation. The results of this additional experiment have been added to the revised manuscript (Lines 180-184) and Appendix A (Fig. A3).

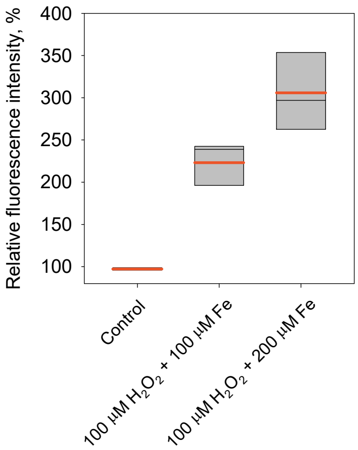

Figure A3 Responsiveness of the CM-H_2DCFDA assay in A549 cells to H_2O_2 (100 µM) with iron ions (100 or 200 µM as $FeCl_2$). Control samples are shown for comparison. Data are based on five independent measurements. The thin black line represents the median, while thick red lines represent the mean. The lower and upper edges of the boxes correspond to 25th and 75th percentiles, respectively.

Response (2)

Zymosan was not used as a positive control in the additional experiment. Zymosan is an agonist of Toll-like receptor 2 (TLR2) and Dectin-1 and is commonly used as a positive control for ROS production induced by receptor-mediated phagocytosis in phagocytic cells such as macrophages (e.g., Landreman et al., 2008). However, epithelial cells generally lack strong phagocytic activity and do not efficiently internalize zymosan particles. In this study, the human alveolar epithelial cell line A549 was used for the CM-H_2DCFDA assay. Therefore, zymosan was not considered an appropriate positive control.

In this study, fluorescence changes in the CM-H_2DCFDA assay were expressed relative to the fluorescence intensity measured immediately after particle exposure. This normalization was applied to reduce well-to-well variability in fluorescence signals arising from differences in probe loading, cell number, and basal oxidative status, and to assess the time-dependent increase in intracellular oxidative activity after particle exposure within the same well. This approach also helps minimize potential optical interference caused by the particles themselves, such as absorption or scattering of excitation or emission light that may affect fluorescence measurements. To clarify the reasons for the normalization procedure, the following sentence has been added to the revised manuscript:

Revised text (2) (Lines 170–172 of the revised manuscript)

“The results were expressed relative to the fluorescence intensity measured immediately after particle exposure. This normalization reduces well-to-well variability due to differences in probe loading and cell number and minimizes particle-related optical interference such as absorption or scattering of excitation or emission light.”

Response (3)

Normalization to an equivalent value based on any specific substance is not considered appropriate because DCFH-based probes do not directly detect a specific ROS species or oxidative pathways, but instead serve as relative indicators of overall intracellular oxidative activity resulting from multiple oxidative processes (Halliwell and Whiteman, 2004; Kalyanaraman et al., 2012). Accordingly, direct quantitative comparison with values reported in other studies is not straightforward, even when normalized to equivalent metrics. To clarify what DCFH-based probes measure, the following sentence in Introduction has been revised as follows:

Revised text (3) (Lines 65–69 of the revised manuscript)

“Although these DCFH-based probes are often assumed to be specific for H_2O_2, that supposition is not accurate. In fact, DCFH-based probes lack specificity for individual reactive species and instead provide a comprehensive assessment of intracellular oxidative activity, including enzyme-mediated, metabolic, and other cellular redox processes (Kalyanaraman et al., 2012; Halliwell and Gutteridge, 2015; Murphy et al., 2022).”

Comment 3 from Referee #1

Lines 220-225: The difference in the contribution from transboundary transport from continental Asia and local anthropogenic emissions to observed both OP^{DTT} and OP^{DCFH} was more significant than seasonal variations. Could you, therefore, quantify the difference in the contribution from transboundary transport and local anthropogenic emissions to OP^{DTT} and OP^{DCFH} at all sites studied?

Response

We thank the reviewer for this important comment. We agree that formal source apportionment, such as positive matrix factorization (PMF), would provide a stronger quantitative basis for evaluating source contributions to OP. However, given the limited number of samples in the present study (n = 14) and the long integration period of each sample (2–3 months), PMF was not expected to provide robust and interpretable source factors for this dataset. Therefore, we did not perform PMF in this revision. Instead, we have added a limitation statement to the revised manuscript clarifying that the source interpretation in this study was based on chemical composition, hierarchical clustering, and backward trajectory analysis, and that the inferred relationships between chemical groups and OP should be interpreted qualitatively rather than quantitatively.

Revised text (Lines 387–391 of the revised manuscript)

"It should be noted that the source interpretation in this study was based on chemical composition, hierarchical clustering, and backward trajectory analysis, rather than formal source apportionment such as positive matrix factorization (PMF). Given the limited number of samples (n = 14) and the 2–3 month integration period of each sample, PMF was not expected to provide robust and interpretable source factors for this dataset. Therefore, the inferred relationships between chemical groups and OP should be interpreted qualitatively rather than quantitatively."

Comment 4 from Referee #1

Lines 281-315: Expression of the concentration of particulate component as a mass fraction (%) is unusual and prevents direct comparison of results with other studies. It is appropriate to replace the mass fraction (%) with commonly used units (i.e., ng/m^3, ug/m^3) or, at least, express the concentration in both ways in parallel.

Response

We thank the reviewer for this comment. We agree that air-volume-based concentrations (e.g., ng m^{-3}, µg m^{-3}) facilitate comparison with previous studies. However, as explained in Lines 119-122, because complete recovery of particles captured inside the cyclone is not possible, the collected particle mass does not directly represent the total particle mass in the sampled air volume. Therefore, accurate atmospheric concentrations cannot be determined. For this reason, we report particulate component concentrations as mass fractions and oxidative potential as mass-normalized values.

Comment 5 from Referee #1

Lines 321-322: K^+ serves as an indicator of biomass burning, not coal combustion.

Response

We thank the reviewer for this important comment. We agree that K^+ serves as an indicator of biomass burning worldwide. Although coal combustion can be a relatively important source of K^+ in regions strongly affected by coal combustion, such as China (Yu et al., 2018), it is not appropriate to describe K^+ as a specific tracer for coal combustion. The manuscript has been revised as follows:

Revised text (Lines 356–363 of the revised manuscript)

"Group 1 includes nssSO_4^{2-}, As, and nssK^+. Sulfate aerosols in East Asia are influenced predominantly by anthropogenic emissions from China (Itahashi et al., 2012). China consumes vast amounts of coal, accounting for approximately half of global coal consumption (Wang and Li, 2016). As in Group 1 is recognized as a typical marker of aerosol particles generated by coal combustion, and atmospheric concentrations in Beijing have been reported to be approximately 20 times higher than those in Tokyo (Okuda et al., 2004). K^+ has also been reported to be emitted in significant amounts from coal combustion in Beijing (Yu et al., 2018). In addition, biomass burning, including agricultural residue burning, is an important source of K^+ in China, particularly during the post-harvest season (e.g., Zhang et al., 2015). Therefore, the sources of the Group 1 species are likely dominated by transboundary transport from China."

Comment 6 from Referee #1

Lines 363-372: It is known that transition metals, quinones and many other particulate components contribute significantly to ROS generation. In this study, only transition metals were analysed. Why did you not also analyse quinones and other organic compounds that are known to contribute to ROS production?

Response

We thank the reviewer for this important comment. We agree that quinones and other organic components in particulate matter, as well as transition metals, can significantly contribute to ROS generation. However, the primary objective of this study was to investigate the relative contributions of emission sources (local urban emissions and long-range transboundary transport) to the intrinsic oxidative potential (OP) of particulate matter. A detailed evaluation of the relative contributions of individual chemical components to OP was beyond the scope of the present study. Therefore, quinones and other organic compounds were not analyzed in this study. Future studies should consider both metal and organic ROS-active components to better understand their relative contributions to the oxidative potential of particulate matter.

Comment 7 from Referee #1

Line 126: Correct $KHPO_4$ to KH_2PO_4

Response

We thank the reviewer for pointing this out. The reagent used with NaH_2PO_4 was dipotassium hydrogen phosphate; therefore, we corrected "$KHPO_4$" to "K_2HPO_4" in the revised manuscript. (Line 144 of the revised manuscript)

Comment 8 from Referee #1

Line 379: Correct Fig. 6 to Fig. 7

Response

Thank you for your careful review. We have changed "Figure 6" to "Figure 7". (Line 435 of the revised manuscript)

Comment 1 from Referee #3: Insufficient engagement with the broader OP spatial gradient and source-attribution literature

The authors are commended for citing key foundational DTT and DCFH-DA literature and for referencing relevant Japan-based work. However, the introduction does not engage with the now-large body of research examining OP spatial gradients in relation to source type, particularly from East Asia and Europe. Studies examining transboundary OP transport to Korea and Japan, and reviews specifically addressing how long-range transport alters OP per unit mass (e.g., the Shiraiwa et al., 2017 review is cited but primarily for the DTT range benchmark) are largely absent from the introduction's framing. I also suggest considering the list below for Asian context OP studies.

The claim that the finding, lower mass-normalized OP for transported versus local aerosol, represents a novel contribution needs to be validated against what has already been shown in the literature. The authors should add a dedicated paragraph in the introduction reviewing what is known about OP spatial variability and source effects on OP from the global and regional literature, and explicitly state what this study adds. Relevant recent literature includes, but is not limited to:

(Borlaza et al., 2022, 2018; Dominutti et al., 2023; Park et al., 2018, 2020; Weber et al., 2021)

Borlaza, L. J., Weber, S., Marsal, A., Uzu, G., Jacob, V., Besombes, J.-L., Chatain, M., Conil, S., and Jaffrezo, J.-L.: Nine-year trends of PM 10 sources and oxidative potential in a rural background site in France, Atmospheric Chem. Phys., 22, 8701–8723, https://doi.org/10.5194/acp-22-8701-2022, 2022.

Borlaza, L. J. S., Cosep, E. M. R., Kim, S., Lee, K., Joo, H., Park, M., Bate, D., Cayetano, M. G., and Park, K.: Oxidative potential of fine ambient particles in various environments, Environ. Pollut., 243, 1679–1688, https://doi.org/10.1016/j.envpol.2018.09.074, 2018.

Dominutti, P. A., Borlaza, L. J. S., Sauvain, J.-J., Ngoc Thuy, V. D., Houdier, S., Suarez, G., Jaffrezo, J.-L., Tobin, S., Trébuchon, C., Socquet, S., Moussu, E., Mary, G., and Uzu, G.: Source apportionment of oxidative potential depends on the choice of the assay: insights into 5 protocols comparison and implications for mitigation measures, Environ. Sci. Atmospheres, 3, 1497–1512, https://doi.org/10.1039/D3EA00007A, 2023.

Park, M., Joo, H. S., Lee, K., Jang, M., Kim, S. D., Kim, I., Borlaza, L. J. S., Lim, H., Shin, H., Chung, K. H., Choi, Y.-H., Park, S. G., Bae, M.-S., Lee, J., Song, H., and Park, K.: Differential toxicities of fine particulate matters from various sources, Sci. Rep., 8, 17007, https://doi.org/10.1038/s41598-018-35398-0, 2018.

Park, M., Wang, Y., Chong, J., Lee, H., Jang, J., Song, H., Kwak, N., Borlaza, L. J. S., Maeng, H., Cosep, E. M. R., Denna, Ma. C. F. J., Chen, S., Seo, I., Bae, M.-S., Jang, K.-S., Choi, M., Kim, Y. H., Park, M., Ryu, J.-S., Park, S., Hu, M., and Park, K.: Simultaneous Measurements of Chemical

Compositions of Fine Particles during Winter Haze Period in Urban Sites in China and Korea, Atmosphere, 11, 292, https://doi.org/10.3390/atmos11030292, 2020.

Weber, S., Uzu, G., Favez, O., Borlaza, L. J. S., Calas, A., Salameh, D., Chevrier, F., Allard, J., Besombes, J.-L., Albinet, A., Pontet, S., Mesbah, B., Gille, G., Zhang, S., Pallares, C., Leoz-Garziandia, E., and Jaffrezo, J.-L.: Source apportionment of atmospheric PM10 oxidative potential: synthesis of 15 year-round urban datasets in France, Atmospheric Chem. Phys., 21, 11353–11378, https://doi.org/10.5194/acp-21-11353-2021, 2021.

Response

We thank the reviewer for this helpful suggestion. We have added a paragraph to the Introduction to provide broader context on OP spatial variability and source effects based on previous studies from Europe and East Asia. The revised text describes that OP differs among source environments, that traffic-related emissions, biomass burning, and metal-rich sources are often more strongly associated with OP than with particle mass, and that secondary inorganic aerosols can contribute substantially to particle mass while making relatively small contributions to DTT-based OP. We also clarified that regional-scale inter-site variations in mass-normalized OP across East Asia remain insufficiently understood in relation to local emissions, transboundary transport, chemical composition, and air-mass history. The added paragraph reads as follows:

Revised text (Lines 73–86 of the revised manuscript)

"The OP of aerosol particles varies among locations and source environments. OP is commonly expressed as either volume-normalized OP (OP_v), which reflects oxidative capacity per unit air volume, or mass-normalized OP (OP_m), which represents intrinsic oxidative activity per unit particle mass. Because particles from different sources can have substantially different OP_m, OP_v is not necessarily explained by particle mass concentration alone. Previous studies in Europe have shown that secondary inorganic aerosol often accounts for a large fraction of particle mass, whereas DTT-based OP is generally more closely related to traffic-related emissions, biomass burning, and metal-rich sources (Daellenbach et al., 2020; Weber et al., 2021; Borlaza et al., 2022; Dominutti et al., 2023). This source dependence is also supported by source-specific toxicity studies showing relatively high oxidative activity for combustion-derived particles, particularly traffic-related emissions, compared with particles such as ammonium sulfate, ammonium nitrate, and road dust (Park et al., 2018b). In East Asia, DTT-based OP has been examined in relation to source characteristics and spatial variability in urban and source-influenced environments, including sites in Korea and China (Borlaza et al., 2018; Yu et al., 2019; Liu et al., 2020b; Wang et al., 2020; Xing et al., 2023; Cheung et al., 2024; Koo et al., 2025). However, how regional-scale inter-site variations in mass-normalized OP across East Asia are related to local emissions, transboundary transport, chemical composition, and air-mass history remains insufficiently understood."

Comment 2 from Referee #3: Absence of formal source apportionment

The authors use backward trajectory analysis combined with hierarchical clustering of chemical composition data to attribute OP to local versus transported sources. This approach is reasonable and the clustering analysis (Figure 9) is informative. However, the interpretive claims, for example, that Group 3 species are unambiguously associated with local emissions, or that transboundary transport dilutes OP through secondary sulfate formation, rely on qualitative reasoning that would be substantially strengthened by a formal source apportionment approach such as positive matrix factorization (PMF), a research-grade technique.

The authors acknowledge that sampling periods of 2–3 months preclude high-resolution temporal analysis, but PMF has been applied successfully to similar 2–3 month bulk samples in the literature. The authors should either (a) perform PMF or an equivalent receptor model on the available chemical data and correlate source factor contributions with OP, or (b) explicitly acknowledge this limitation and discuss how it affects the confidence with which source–OP relationships can be drawn from the clustering approach alone.

Simply noting that the sampling period is long (lines 342–343) is insufficient as a methodological justification. The source attribution of Cu and Zn to waste fly ash rather than brake dust (lines 344–347) also deserves greater scrutiny: the Cu/Zn ratio argument depends on the reference values chosen, and the authors should note that traffic-related non-exhaust emissions (including brake dust and tire wear) are also commonly reported at urban sites in Japan, and that a mixed contribution is likely.

Response

We thank the reviewer for this important comment. We agree that formal source apportionment, such as positive matrix factorization (PMF), would provide a stronger quantitative basis for evaluating source contributions to OP. However, given the limited number of samples in the present study (n = 14) and the long integration period of each sample (2–3 months), PMF was not expected to provide robust and interpretable source factors for this dataset. Therefore, we did not perform PMF in this revision. Instead, we have added a limitation statement to the revised manuscript clarifying that the source interpretation in this study was based on chemical composition, hierarchical clustering, and backward trajectory analysis, and that the inferred relationships between chemical groups and OP should be interpreted qualitatively rather than quantitatively.

We also agree that the attribution of Cu and Zn to waste fly ash should be interpreted more cautiously. We have revised the discussion to note that possible sources of Cu and Zn include both fly ash from waste incineration and traffic-related non-exhaust emissions, such as brake wear and tire wear. Although the observed Cu/Zn ratio was closer to that of waste fly ash than to that of brake dust, we now state that traffic-related non-exhaust emissions cannot be completely excluded because this interpretation is based on elemental ratios and clustering analysis rather than formal source apportionment.

The corresponding discussion has been revised as follows:

Revised text (Lines 377–391 of the revised manuscript)

"Group 3b species comprise metal elements. In urban atmospheres, a wide variety of anthropogenic activities emit metal-containing fine particles. Although the metals in Group 3b were classified into the same group based on clustering analysis, their actual sources might vary widely because of the long sampling periods (2–3 months) in this study. Cu and Zn exhibited a particularly strong correlation ($r = 0.91$), indicating that they might have originated from similar or co-occurring sources. Possible sources of Cu and Zn include fly ash from waste incineration and traffic-related non-exhaust emissions, such as brake wear and tire wear. According to Iijima et al. (2009), Cu/Zn ratios differ markedly between waste fly ash and brake dust (0.07 and 14, respectively). The average Cu/Zn ratio observed in our samples was approximately 0.2, which is closer to that of waste fly ash than to that of brake dust, suggesting that waste fly ash might be an important contributor to Cu and Zn in these samples. However, traffic-related non-exhaust emissions cannot be completely excluded, because this interpretation is based on elemental ratios and clustering analysis rather than formal source apportionment.

It should be noted that the source interpretation in this study was based on chemical composition, hierarchical clustering, and backward trajectory analysis, rather than formal source apportionment such as positive matrix factorization (PMF). Given the limited number of samples ($n = 14$) and the 2–3 month integration period of each sample, PMF was not expected to provide robust and interpretable source factors for this dataset. Therefore, the inferred relationships between chemical groups and OP should be interpreted qualitatively rather than quantitatively."

Comment 3 from Referee #3: Underreporting of CM-H_2DCFDA assay methodology and dose justification

CM-H_2DCFDA protocol (lines 141–157), while adequate in outline, omits several details that are necessary for reproducibility and scientific interpretation. First, the particle mass concentration used in the exposure (100 µL of a 2 mg/20 mL suspension = 100 µg/mL) is never stated explicitly, readers must calculate it from the dispersed information in the text. Second, no cytotoxicity assessment is reported at this dose. A549 cell viability under particle exposure should be verified (e.g., by LDH release, MTT, or trypan blue), particularly for the high-OP Yokohama samples; without this, it is unclear whether elevated fluorescence in some samples reflects genuine ROS production or cell membrane disruption. Third, the decision to express $OP^{DDMH}m$ as percentage of fluorescence at 6 hr relative to immediately post-exposure is unconventional, most DCFH-based studies normalize to vehicle control or unexposed cells at the same time point, making inter-study comparison difficult. The authors should justify this normalization choice and discuss how it affects comparability with the literature values they cite. Fourth, a dose–response relationship for at least one representative sample would strengthen confidence that the measured fluorescence differences among sites reflect genuine OP differences and not saturation or threshold effects.

Response (1)

We thank the reviewer for this helpful comment. We have revised the sentence to explicitly state the concentration of the suspension (100 µg/mL). The revised text now reads: "yielding a final particle concentration of 100 µg/mL," (Line 162 in the revised manuscript).

Response (2)

To assess potential cytotoxicity under the exposure conditions used for the CM-H_2DCFDA assay, A549 cell viability was evaluated using a water-soluble tetrazolium salt-1 (WST-1) assay for all particle samples collected in this study. The results are presented in Figure A2 in Appendix A. Mean cell viability remained above 80% for all particle samples under the exposure conditions used for the CM-H_2DCFDA assay, suggesting that the contribution of cytotoxic effects to the measured fluorescence responses was likely limited. The corresponding description has been added to Lines 175–180 of the revised manuscript.

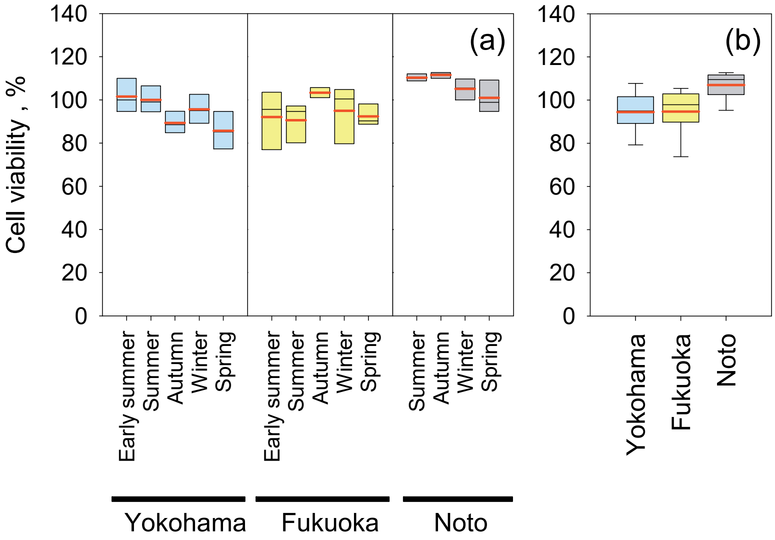

Figure A2. Cell viability of A549 cells exposed to particle suspensions under the same conditions used for the CM-H2DCFDA assay. Cell viability was evaluated using the WST-1 assay after 6 hr exposure to particles at a concentration of 100 µg/mL. Values are expressed relative to the control. (a) Mean cell viability for individual particle samples based on four independent measurements. (b) Mean cell viability grouped by sampling location. The thin black line represents the median, while thick red lines represent the mean. The lower and upper edges of the boxes correspond to the 25th and 75th quartiles, respectively.

Response (3)

In this study, fluorescence changes in the CM-H_2DCFDA assay were expressed relative to the fluorescence intensity measured immediately after particle exposure. This normalization was applied to reduce well-to-well variability in fluorescence signals arising from differences in probe loading, cell number, and basal oxidative status, and to assess the time-dependent increase in intracellular oxidative activity after particle exposure within the same well. This approach also helps minimize potential optical interference caused by the particles themselves, such as absorption or scattering of excitation or emission light that may affect fluorescence measurements. In contrast, normalization to the control may not adequately correct for particle-specific optical interference, because absorption or scattering effects caused by the particles are not represented in the control wells. To clarify the reasons for the normalization procedure, the following sentence has been added to Section 2 of the revised manuscript:

Revised text (3) (Lines 170-172 in the revised manuscript)

“The results were expressed relative to the fluorescence intensity measured immediately after particle exposure. This normalization reduces well-to-well variability due to differences in probe loading and cell number and minimizes particle-related optical interference such as absorption or scattering of excitation or emission light."

Response (4)

To partially address the reviewer's concern regarding potential saturation or threshold effects, an additional experiment was conducted to confirm the responsiveness of the CM-H_2DCFDA assay. Because the remaining amount of particle samples was insufficient for additional validation experiments, a chemically defined ROS-generating system consisting of H_2O_2 and iron ions was used instead of the original samples. A549 cells loaded with CM-H_2DCFDA were exposed to a mixture of H_2O_2 (100 µM) and iron ions (100 µM or 200 µM). This exposure concentration-dependently increased fluorescence intensity compared with the control, with the maximum value reaching approximately 300% of the initial value, confirming that the assay system used in this study responds to intracellular ROS generation over a broad range of fluorescence intensities. The results of this additional experiment have been added to the revised manuscript (Lines 180-184 in the revised manuscript) and Appendix A (Fig. A3).

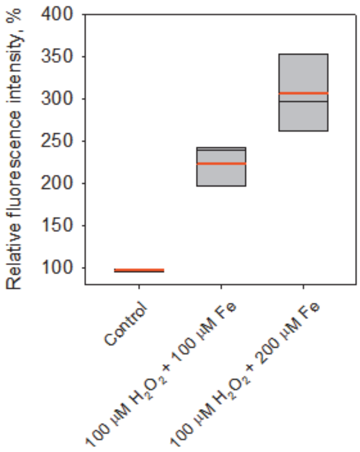

Figure A3 Responsiveness of the CM-H_2DCFDA assay in A549 cells to H_2O_2 (100 µM) with iron ions (100 or 200 µM as $FeCl_2$). Control samples are shown for comparison. Data are based on five independent measurements. The thin black line represents the median, while thick red lines represent the mean. The lower and upper edges of the boxes correspond to the 25th and 75th percentiles, respectively.

Comment 4 from Referee #3: Seasonal OP patterns require more complete mechanistic explanation

The authors note that seasonal variations in OP$^{\mathrm{DDM}}$m and OP$^{\mathrm{DDHM}}$m were smaller than inter-site differences, and attribute this to the counteracting effects of seasonal air mass origin and boundary layer height (lines 246–263). This is a plausible explanation, and the reference to Hara et al. (2021) supports it. However, the observation that OP$^{\mathrm{DDM}}$m peaks in autumn at all three sites (line 211) is interesting and not fully explained. Autumn in Japan is associated with increased continental transport (as shown in Figures 5 and 6) but also with biomass burning in continental Asia (crop residue burning, particularly in northeastern China, is well documented during September–November). The contribution of biomass burning to autumn OP, including via the levoglucosan/potassium pathway, deserves explicit consideration, especially since the nssK$^+$ is classified in Group 1 (continental transport) and shows only weak correlation with OP. The authors should address whether autumn peaks in OP$^{\mathrm{DDM}}$m might reflect a modest biomass burning signal superimposed on local emissions, and whether the available chemical tracers are sufficient to detect such a contribution given the 2–3 month sampling resolution.

Response

We thank the reviewer for this insightful comment regarding the possible contribution of biomass burning to the autumn enhancement of OP$^{\mathrm{DTT}}_{\mathrm{m}}$. As suggested, post-harvest crop residue burning in East Asia might contribute to the seasonal increase in OP through long-range transport of biomass-burning-related aerosols. To further examine this possibility, we additionally analyzed the seasonal variation of nssK$^+$ concentrations and added the results as Figure A4 in Appendix A. nssK$^+$ concentrations were elevated during autumn at all sites, with the highest levels observed in Yokohama and Noto and relatively high levels also observed in Fukuoka. These observations are consistent with a possible contribution of biomass-burning-related aerosols during autumn. However, because specific biomass-burning tracers such as levoglucosan were not measured and the aerosol samples were collected over relatively long integration periods (2–3 months), the contribution of biomass burning could not be quantitatively evaluated in this study. The corresponding discussion has been added to the revised manuscript (Lines 241–245 in the revised manuscript).

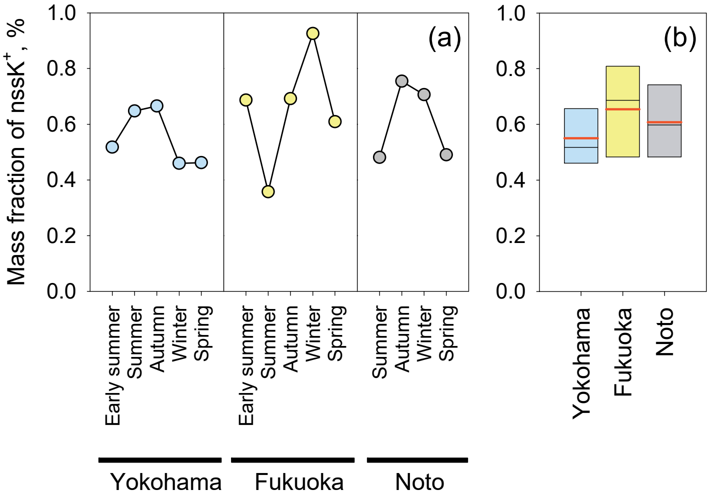

Figure A4. Mass fraction of nssK+: (a) mass fractions for individual particle samples and (b) mean values for the Yokohama, Fukuoka, and Noto sites. The thin black line represents the median, while thick red lines represent the mean. The lower and upper edges of the boxes correspond to the 25th and 75th percentiles, respectively.

Comment 5 from Referee #3: Conditions limiting the DTT–DCFH correlation should be addressed more explicitly

The strong correlation between $OP^{DTT}m$ and $OP^{DCFH}m$ (r = 0.81) across all sites is a useful and interesting result, and the authors compare it appropriately to prior studies. However, the discussion focuses primarily on why the correlation holds rather than examining the boundary conditions under which it might break down specifically for Japanese aerosols. Given the unique source mixture in Japan, characterized by strong transboundary transport of sulfate-rich, metal-poor aerosols mixed with local combustion emissions, it would be informative to discuss whether the correlation is driven largely by the between-site gradient (i.e., the Yokohama points anchoring the high end) or whether it also holds within-site across seasons. A within-site correlation analysis would address this and would add interpretive value. Additionally, the discussion notes that the DCFH assay uses CM-H_2DCFDA rather than the conventional DCFH-DA probe (lines 57–70), and the authors correctly describe differences in cellular retention and ROS specificity. However, the consequence of this probe choice for the DTT–DCFH correlation comparison with prior studies that used conventional DCFH-DA should be discussed: since CM-H_2DCFDA may have different sensitivity profiles for specific ROS, the comparability of r-values across studies is not guaranteed.

Response (1)

We thank the reviewer for this helpful comment. We additionally examined within-site relationships between OP_m^{DTT} and OP_m^{DCFH} to assess whether the overall correlation was driven by between-site differences. Within-site correlations varied considerably among sites (r = 0.28, −0.68, and −0.16 for Yokohama, Fukuoka, and Noto, respectively), suggesting that the overall correlation between OP_m^{DTT} and OP_m^{DCFH} was largely influenced by the between-site gradient rather than by consistent seasonal covariation within each site. The following discussion has been added to the revised manuscript.

Revised text (1) (Lines 445–449 in the revised manuscript).

"However, Figure 11 also indicates that the relationship between OP_m^{DTT} and OP_m^{DCFH} was less apparent within individual sites, suggesting that the overall correlation was influenced primarily by differences among sites rather than by consistent seasonal covariation within each site. This suggests that a clear correlation between the two OP metrics might be detectable only when the dataset covers a sufficiently wide range of OP values."

Response (2)

We also agree that caution is warranted when comparing the correlation coefficients observed in this study with those reported in previous studies using the conventional DCFH-DA probe. CM-H_2DCFDA differs from DCFH-DA in intracellular retention characteristics and might exhibit different sensitivity profiles toward specific ROS species. Therefore, direct comparison of DTT–DCFH correlation coefficients among studies should be interpreted with caution. The corresponding caveat has been added to the revised manuscript.

Revised text (2) (Lines 465–471 in the revised manuscript)

“Unlike our study, those earlier studies analyzed water suspensions or aqueous extracts of aerosol particles collected on filters, used the conventional DCFH-DA probe rather than CM-H_2DCFDA, and employed rat alveolar macrophages instead of A549 cells. Therefore, direct comparison of correlation coefficients among studies should be interpreted with caution, and the specific factors influencing OP values observed in those studies might differ from ours. Nonetheless, these previous findings, together with the significant overall correlation observed in the present study, suggest that the two assays can respond similarly to broad spatial differences in aerosol oxidative properties associated with anthropogenic emissions.
”

Comment 6 from Referee #3 Comparison with published OP values from East Asia needs greater context

The authors compare their $OP^{DDM}m$ values with those from Beijing (Yu et al., 2019) and from the Fukuoka site in prior work (Nishita-Hara et al., 2019; Fujitani et al., 2023), and explain differences in terms of assay conditions. This is handled well. However, given the stated aim of positioning the study in the context of transboundary transport from continental Asia, comparison with OP data from Korean sites, which sit geographically between China and Japan and thus represent an intermediate point along the transport pathway, would substantially strengthen the narrative. Published OP data from Seoul and other Korean cities are available in recent literature. Similarly, the discussion of the 'dilution by secondary sulfate' mechanism (lines 355–357, 419–421) is invoked as a plausible explanation for the OP decrease during transport, but this mechanism has been discussed in prior OP literature and should be cited more explicitly. The authors should expand the geographic comparison and engage more directly with the dilution-versus-chemical-transformation debate in the OP transport literature.

Response

We thank the reviewer for this helpful suggestion. We agree that published OP data from Korea provide important context for interpreting the present results in relation to transboundary transport from continental Asia.

For direct numerical comparison in the Discussion, we used Kim et al. (2024), who reported a mean mass-normalized OP_m^{DTT} value of 27.5 pmol min^{-1} μg^{-1} for $PM_{2.5}$ collected in Chuncheon, South Korea. This value was comparable to the level observed at Noto in the present study but lower than those observed at Yokohama and Fukuoka. We used Kim et al. (2024) for this comparison because its reported mass-normalized OP_m^{DTT} value was more directly comparable to the OP_m^{DTT} values in the present study. Borlaza et al. (2018) was not used for direct numerical comparison because the DTT concentration and water-extraction procedure differed substantially from those used in the present study.

We also revised the discussion of the possible OP decrease during transport by adding citations to previous OP studies that discuss the dilution mechanism, in which secondary inorganic aerosols such as sulfate increase particle mass but do not contribute proportionally to DTT-based OP. The revised Discussion paragraph reads as follows:

Revised text (Lines 392–410 in the revised manuscript):

"Figure 10 presents correlation coefficients of OP_m^{DTT} and OP_m^{DCFH} with the mass fractions of individual chemical species. Both OP metrics exhibited negative correlations with most Group 1 chemical species ($r = -0.57$–0.01), suggesting that the mass-normalized OP of fine particles in Japan tends to decrease as the relative contribution of transboundary transport increases. In Beijing, China, Yu et al. (2019) measured OP_m^{DTT} of the aqueous extract of fine particles collected over the course of one year using the DTT assay under similar conditions to those used in this study: measuring the loss of 100 μM DTT in 0.10 M phosphate buffer at pH7.4. The mean (standard deviation) OP_m^{DTT} value was 130 (100) pmol min^{-1} μg^{-1}, which was much higher than the values observed in Japan in this study. Therefore, the mass-normalized OP of fine aerosol particles transported from continental Asia might decrease during regional transport to Japan, probably partly because of dilution by secondary particles, such as sulfate, that increase particle mass but do not necessarily contribute proportionally to aerosol OP, consistent with previous source-apportionment studies showing that secondary inorganic aerosol can contribute substantially to particle mass, whereas DTT-based OP is more strongly associated with traffic-related emissions, biomass burning, and metal-rich or mineral-dust-related sources (Weber et al., 2021; Borlaza et al., 2022). Kim et al. (2024) reported a mean (standard deviation) OP_m^{DTT} value of 27.5 (11.8) pmol min^{-1} μg^{-1} for $PM_{2.5}$ collected in Chuncheon, South Korea. Although their OP_m^{DTT} value was also determined for particle suspensions including insoluble components, as in the present study, the comparison should be interpreted with caution because the particles were extracted from filter samples using methanol and might not have been completely recovered from the filters. This value was comparable to the level observed at Noto in this study but lower than those observed at Yokohama and Fukuoka, despite the more urbanized setting of Chuncheon compared with the Noto site. The relatively low OP_m^{DTT} value at Chuncheon might suggest that dilution of redox-active aerosol components had already occurred before or during transport to Korea."

Comment 7 from Referee #3: Minor issues

Several minor issues should be corrected. (a) Line 81: 'intercellular' should read 'intracellular'. (b) Line 465: 'Rditing' should read 'Editing'. (c) Line 382 refers to 'Figure 6' when describing the order of transition metal concentrations, this appears to be a reference error; the relevant data are in Figure 7 or Figure 8. Please verify all figure cross-references. (d) The abstract states that $OP^{DDHM}m$ was correlated 'strongly' with carbonaceous components and transition metals, the word 'strongly' should be qualified or replaced with the actual correlation range (r = 0.47–0.84 for the relevant species in Figure 10) to be accurate.

Response

Thank you for your careful review. All of the issues you pointed out have been corrected, including typographical errors, the figure reference, and the figure cross-references. We have also replaced “strongly” in the abstract with the corresponding correlation range (r = 0.47–0.84).