Authors' response to RC#1

General comment: In this work, the authors describe the development and the measurements of oxidative potential of PM by a semi-automated system compared to routine approach using DTT assay. Furthermore relationships between this oxidative potential and season as well to PM mass are then investigated. The routine and practicable measurement of oxidative potential of PM is of great importance because of the possible roles in both aerosol ageing and health impacts. In consequence this work is clearly important and within the scope of AMT. My main concerns with this manuscript are mostly related to the focus of the manuscript. In my opinion the authors should give more detailed information on the semi-automated system compared to the routine system (e.g. saving time and costs, handling, as well applicability in routine analysis). Furthermore, we appreciate the experiments conducted in order to determine precision and accuracy of the system, but the uncertainty of the entire system is still not quantified, neither is the uncertainty of the experimental protocol performed manually. Finally, the correlation analysis to mass and a discussion to health relevance should be reduced/rephrased as long as no input of the correlations of the oxidative potential to chemical composition and health endpoints are given.

Authors' response:

1) *Based on the reviewer suggestion, more discussion on the semi-automated system compared to manual method were added:*

Page 7248 line 24: It generally takes at least five hours for a two-person team to analyze five samples manually, limiting the total number of samples that can be analyzed daily..

Page 7249 line 7: "The semi-automated system can run for 24 hours unattended and can also be monitored remotely."

2) *The uncertainty of the semi-automated system was in fact assessed by the variability of positive control (9,10-phenanthraquinone) through the whole analysis period, see page 7257 line 16 "The system remained fairly consistent throughout the analysis with reasonably small variability for both positive controls (CV = 15%) and blanks (DI blanks: CV = 28.1%; field blanks: CV = 26.7%)."*

   The uncertainty of both semi-automated system and manual method were also assessed at the same time by five replicates of 9,10-phenanthraquinone solutions. See page 7256 line 20: "The DTT consumption rate obtained from the automated system (mean ± stdev of 0.77 ± 0.03 nmol/min, CV= 4.24%) was very close to that from the manual operation (0.74 ± 0.03 nmol/min, CV= 3.97 %)." Therefore, the authors considered the uncertainty of both methods were suitably quantified.

3) *Section 3.2.4 – the discussion on oxidative potential and PM2.5 and health relevance was reduced.*

Specific comments:

- line 39: Is the CV for your standard really higher than for ambient samples? This is unusual and I suggest thinking about an alternative standard and name it here positive control!

Authors' response: Replaced "standard" with "positive control" in the entire manuscript.

-line 45, 46: If the DTT activity is well correlated to PM2.5 mass why should I measure with DTT assay?

Authors' response: As we stated in section 3.2.4: "The overall correlation between water-soluble DTT activity and PM2.5 mass concentration observed in this study may help explain, at least in part, some of these associations. However the varying degree of correlation between DTT activity and PM2.5 mass at different sites and seasons suggests that there are additional advantages of including PM oxidative potential in health studies, rather than relying on PM mass alone." For example, although DTT activity and PM2.5 mass are correlated at a specific site over one-month sample periods, the slope differs between sites and seasons indicating contributions from different sources. We believe not all PM components are DTT active and thus measuring DTT activity provides a means of helping to identify aerosol sources that may be especially toxic.

And why are you suggesting that regional sources and not long term transport might be the reason for the variance? In the following sentence this is indirect suggested by the seasonality. Please be more specific and clarify this sentence/your statement.

- line 372: Can you please explain exactly why do you think the southeast activity is related to regional and not local/single sources?

- line 406-408: Please give also here reasons why this stands for regional sources? What about e.g. with long term transport?

Authors' response: The reviewer raised the question why the spatial homogeneity of the DTT activity per volume basis in southeast US indicates regional sources rather than local/long-term transport sources three times in the comments listed above. The authors would like to address them all at the same time. PM samples analyzed for DTT activity in this study were collected from contrasting environment including rural, urban and roadside. PM sources at these sites are considered very different, for example, roadside would have a stronger impact from vehicle emissions than the rural site. However, the DTT activity per volume basis is quite spatial uniform regardless of sites which indicates that there are regional sources impacting the sampling sites and that the DTT activity based on filters is not dominated by a local unique source. Otherwise, we would expected a spatially heterogeneous distribution of the DTT activity in this region. Spatial uniformity suggests either a region source (such as SOA from dispersed emissions, BVOCs) or a well-mixed atmosphere of emissions from distant sources (e.g., long range transport).

Edited at page 7259 line 21 "Relatively low levels of the CODs (<0.25) (Table. 3) found for both DTTv and DTTm at paired-sites indicate spatial homogeneity of water-soluble aerosol oxidative potential in the region, suggesting dominant DTT activity sources are regional in nature, such as SOA (secondary organic aerosol) or a well-mixed atmosphere of emissions from distant sources (e.g., long range transport)."

- line 87: What is missing in this section is ROS generation after inhalation the main exposure pathway and e.g. activation of immune system like macrophage response finally also maybe leading to ROS. I would suggest including a short passage on this.

Authors' response: Edited page 7247 line 6 "The mechanisms underlying these associations are not entirely clear but reactive oxygen species (ROS) have been identified as signaling molecules that induce oxidative stress, causing cell damage (Nel, 2005; Gurgueira et al., 2002). ROS can either be adsorbed on inhaled particles or generated in vivo by targets cells such as airway epithelial cells and macrophages (Li et al., 2003; Nel et al., 1998)."

- line 97: This a very general comment can you be a bit more precise and give example for time needed for analysis please?

Authors' response: Added example page 7248 line 24: "It generally takes at least five hours for a two-person team to analyze five samples manually, limiting the total number of samples that can be analyzed daily."

- line 107: You mentioned a manual protocol, is this lab internal or is it based on published literature, if so please refer to it.

Authors' response: It is based on a published literature, reference added.

- line 189: Please rephrase, the wording is a bit misleading! 3.5 mL with a concentration of 40 µg/mL represent a total PM mass of 120 µg.

Authors' response:
Edited page 7252 line 17 as follows: For example, the total PM mass in the incubation vial for a sample with liquid concentration of 40µg/mL would be 140 µg (3.5 mL × 40µg/mL).

-line 205: If possible please provide a map and maybe also a picture of the trailer in the supplement, that would makes it easier for the reader to get an impression of the investigation/area

Authors' response: A map of sampling site and a picture of the trailer were added in the supplement.

- line217-231: Although the extraction efficiency of the water-soluble substances is the relevant/crucial factor within your system and we assume it to be fairly good, we propose to give some estimate on the total extraction efficiency (includ-ing that of particles) of the applied approach.

Authors' response: There is unfortunately no perfect way to accurately determine the extraction efficiency of total water soluble PM that contributes to the DTT activity since they likely include many unknown compounds (Bein et al,. 2014). We could estimate the extraction efficiency for water soluble organic carbon (WSOC), which comprise a large fraction of water soluble PM2.5 by looking at the water-to-sample ratio (volume of extraction liquid/volume of air sampled). The water-to-sample ratio in our approach is 0.26 cm3/m3, which is near what has been considered the optimal water-to-sample ratio (0.1 cm3/m3) suggested by Psichoudaki and Pandis (ES&T, 2013) for full extraction of WSOC.

- line 226: Please specify the sonication procedure!

Authors' response: page 7254 line 7 edited as follows:
For the water extraction procedure, portions of the filters (about 1/28 area of the Hi-Vol filter, three one-inch diameter punches) were punched and extracted in 15 mL of DI water in sterile polypropylene centrifuge tubes (VWR International LLC, Suwanee, GA, USA) by sonication using an Ultrasonic Cleanser (VWR International LLC, West Chester, PA, USA) for 30 minutes.

- line 252: Insert space in front of Kumagi et al.

Authors' response: space inserted.

- line 265: So please state, LOD is 20 µg/mL correct?

Authors' response: The LOD in this case, defined as three times the standard deviation of DI blanks, is 0.31 nmol/min. Note that this value represent the consumption rate of DTT in unit of nmol/min. As stated in the manuscript, expressing the LOD in terms of PM liquid concentration could not be achieved as it depends on various factors. 20 µg/mL is a suggested liquid concentration for the instrument in order to yield reliable results above LOD.

- line 252: Insert space in front of mL

Authors' response: Space inserted.

- line 282: Please give full name for abbreviation of PQN

Authors' response: Full name inserted.

- line 287: Please give the CVs

Authors' response: The slope (1.08±0.12) and intercept (-0.02±0.03) were obtained from an orthogonal fit between the automated system and manual method. The correlation coefficient (r2) is 0.92. There is no CVs information associated with this linear fitting.

- Line 154: Is there any DTT left in the system after self-cleaning? We recommend mentioning that the carry-over has been quantified and attach the data in the supplement.

Authors' response: The incubation and reaction vial were cleaned three times with DI water after each sample. See figure 3(a) B_1, B_2, B_3, B_4, and B_5, after each data measurement, absorbance at 412 nm equals absorbance at 700 nm, resuming the absorbance to the baseline, which indicates no carry-over issue in the system after self-cleaning.

- line 305: In abstract the CV for standard is given with 12% here 15%?

Authors' response: page 7246 line 12, changed to 15% in the abstract.

- line 322: Please check/insert space in front of the authors

Authors' response: Space inserted.

- line 330: I agree with the authors but this sentence does not provide any extra information an is/should be in the conclusion sector.

Authors' response: page 7258 line 21, sentence "Overall, these results show the utility of the automated system for providing a comprehensive assessment of the aerosol oxidative properties." deleted.

- line335: Please give some information on the statistical analysis normal distributed, no heteroscedasticity, post hoc..used?

Authors' response: The statistical analysis was done assuming normally distributed data.

- line370: I fully agree but of certain interest would be here also to have some chemical component information. Maybe just briefly you could provide some information at least to some elements or group of elements etc. that later will be published in Verma et al. as you mentioned.

Authors' response: page 7260 line 18 added: "For example, DTT activity correlates with water soluble organic carbon (WSOC) in summer (R>0.67), but correlates better with brown carbon in winter (R>0.78). Further investigation on identifying the specific sources and aerosol chemical components linked to PM oxidative potential was discussed in Verma et al., (2014)."

- line 377: This is a very unspecific statement and the listed publication are of course fine but providing some more detailed information of PM related Oxidative stress and subsequent endpoints like oxidative DNA damage, increasef inflammatory markers etc. at least listing some review articles ore even books would be nice here. Otherwise it becomes not clear how the DTT reactivity is related to health effect endpoints.

Authors' response:
Page 7260 line 21, added detailed endpoints as follows:
Studies have shown a correlation between PM2.5 mass and health end-points, such as HRV, blood neutrophils, and lipids changes (Tong et al., 2012), increased oxidative DNA damage and nucleobases (Peter and Steffen, 2010), and cardiovascular mortality (Gholampour et al., 2014).

Reference:
Gholampour, A., Nabizadeh, R., Naseri, S., Yunesian, M., Taghipour, H., Rastkari, N., Nazmara, S., Faridi, S., and Mahvi, A. H.: Exposure and health impacts of outdoor particulate matter in two urban and industrialized area of Tabriz, Iran, Journal of Environmental Health Science and Engineering, 12, 27, doi:10.1186/2052-336X-12-27, 2014.

Li, N., Sioutas, C., Cho, A. K., Schmitz, D., Misra, C., Sempf, J., Wang, M., Oberley, T., Froines, J., and Nel, A.: Ultrafine particulate pollutants induce oxidative stress and mitochondrial damage, Environmental Health Perspectives, 111, 455-460, 2003.

Nel, A.: Air pollution-related illness: effects of particles, Science, 308, 804-806, 2005.

Nel, A. E., Diaz-Sanchez, D., Ng, D., Hiura, T., and Saxon, A.: Enhancement of allergic inflammation by the interaction between diesel exhaust particles and the immune system, Journal of Allergy and Clinical Immunology, 102, 539-554, http://dx.doi.org/10.1016/S0091-6749(98)70269-6, 1998.

Peter, M., and Steffen, L.: Oxidative damage to DNA and Lipids as biomarkers of exposure to air pollution, Environmental Health Perspectives, 118, 1126–1136, 2010.

Psichoudaki, M. and Pandis, S.N., Atmospheric Aerosol Water-Soluble Organic Carbon Measurement: A Theoretical Analysis, Environ. Sci. Technol., 47 (17), pp 9791–9798 DOI: 10.1021/es402270y, 2013.

Tong, H., Rappold, A. G., Diaz-Sanchez, D., Steck, S. E., Berntsen, J., Cascio, W. E., Devlin, R. B., and Samet, J. M.: Omega-3 fatty acid supplementation appears to attenuate particulate air pollution-induced cardiac effects and lipid changes in healthy middle-aged adults, Environ Health Perspect, 120, 952-957, 2012.

Verma, V., Fang, T., Guo, H. Y., King, L. E., Edgerton, E. S., Weber, R. J. (accepted): Reactive oxygen species associated with water-soluble PM2.5 in the southeastern United States: spatiotemporal trends and source apportionment, , Atmos. Chem. Phys. Discuss., 14, 19625-19672, doi:10.5194/acpd-14-19625-2014, 2014.

Authors' response to RC#2

This paper describes an automated method for measuring aerosol oxidative load using an on-line DTT assay. The system is complicated but effective and provides the ability to generate kinetic curves. Previous concerns regarding the introduction and methods descriptions have been suitably addressed now.

Authors' response: There is no issue needs to be addressed for this comment.

Authors' response to RC#3

This article summarized a semi-automated system and its application for quantifying the oxidative potential of ambient particles in aqueous extracts using the dithiothreitol (DTT) assay. Based on the authors' claim, this method is robust, efficient and more importantly time-saving. However, no information is provided on the conventional manual system or a comparison of the two methods was included. In terms of validation of the method, the authors' initiatives are commendable (e.g. conducting tests to determine instrument response, limit of detection (LOD), precision and accuracy).The spatial and seasonal variability analysis approach raises some confusion, specifically on how these correlations help to identify what types of sources are contributing. Including rationale behind using different parameters e.g. DTTv DTTM will be helpful for readers. In the result section, statement should be well supported by direct experimental results and any kind of presumption should be backed by examples from literature. More information is needed on the discussion towards health end-points and oxidative potential. Specific comments about the article are addressed below.

Authors' response:

1) *Based on the reviewer suggestion, more discussion on the semi-automated system compared to manual method were added:*

Page 7248 line 24: It generally takes at least five hours for a two-person team to analyze five samples manually, limiting the total number of samples that can be analyzed daily.

Page 7249 line 7: "The semi-automated system can run for 24 hours unattended and can also be monitored remotely."

2) *The logic of using DTTv and DTTm is added to section 3.2.3. See detailed authors' responses to the reviewer's 6 and 7 comments below.*
3) *The reviewer suggests more information on the discussion of health end-points and oxidative potential should be added. However, since 1) the focus of this work is mainly on the development of a semi-automated system and its application in a large number of filter analyses, and 2) the associations of the PM-driven oxidative stress and health endpoints has been extensively discussed in the existing literature (ref.) Investigation of the DTT-measured oxidative potential and health effects is a topic of our future paper and therefore, the authors consider it inappropriate to discuss it extensively here.*

Comments:

1. Page 7354, section 2.3.2, line 13-14, The sentence 'The other fraction was reserved for other chemical analysis.' is redundant. Otherwise, please explain what are included in the 'other chemicals'.

Authors' response: Edited as "The other fraction was reserved for other chemical analysis including water-soluble organic carbon and brown carbon."

2. Section 2.3.2, what is the recovery efficiency of the filter extraction process?

Authors' response: There is unfortunately no perfect way to accurately determine the extraction efficiency of total water soluble PM that contributes to the DTT activity since they likely include many unknown compounds (Bein et al,. 2014). We could estimate the extraction efficiency for water soluble organic carbon (WSOC), which comprise a large fraction of water soluble $PM_{2.5}$ by looking at the water-to-sample ratio (volume of extraction liquid/volume of air sampled). The water-to-sample ratio in our approach is 0.26 cm^3/m^3, which is near what has been considered the optimal water-to-sample ratio (0.1 $cm^3/m3$) suggested by Psichoudaki and Pandis (ES&T, 2013) for full extraction of WSOC.

3. Section 3.1.4 line 17-18, what is the experimental protocol of the manual method mentioned here? Since the goal of this paper to portrait the robustness of the automated method over the manual one, a side by side comparison or detailed information may be helpful.

Authors' response: The experimental protocol for the automated system was same as the traditional manual approach. A reference of the traditional manual method was added: Page 7256 line 18: "The system was validated for accuracy by comparing the DTT activity of both positive controls and ambient PM samples obtained from the automated approach with that from the same experimental protocol performed manually (Cho et al., 2005)."

The information on the advantage of automated system over manual method in terms of time and labor saving was added into the main text as below:

Page 7248 line 24: It generally takes a two-person team five hours to process five samples manually, limiting the total number of samples that can be analyzed daily.

Page 7249 line 7: "The semi-automated system can run unattended analyses for 24 hours and can be monitored remotely."

A table summarizing the comparison of manual and semi-automated methods are added in the supplemental information as follows:

Table S1. Comparison of semi-automated DTT assay system to the traditional manual method.

	Traditional manual method	**Semi-automated method**
Labor involved	Yes	No
Numbers of samples analyzed daily	5-8	24
Time for each sample, h	1	1
Remote control	No	Yes
Reaction Volume, mL	1	5
Reaction Vial	Multiple vials	Single vial
Limit of Detection, nmol/min	0.26 (N=5)	0.31 (N=37)

4. Section 3.2, page 7257, line16-18, why the CV for the blanks are relatively higher in comparison with the standards?

Authors' response: Since blanks' levels are close to the LOD of the system, the uncertainty associated with blanks are higher which leads to higher CVs.

5. Section 3.2.2, page 7258, line 21-22, how the data/result interpretation helps to prove that the developed automated system provides comprehensive assessment? Is it based on the fact that a larger set of data was analyzed? The same objective could have been accomplished using manual protocol except longer time would be needed. Again a comparison of manual and automated system would be relevant.

Authors' response: Deleted: "Overall, these results show the utility of the automated system for providing a comprehensive assessment of the aerosol oxidative properties".

6. Section 3.2.3, what is the rationale behind using both DTTv and DTTm to evaluate seasonal variability and then concluding (line18-20, page 7259) that "higher seasonal differences in DTTm may suggest that the specific chemical that contributes to the oxidative potential of particles varies between seasons and originate from different sources."

7. Section 3.2.3, both DTTV and DTTm were used to explain spatial variability and it was justified as both these parameters yielded low COD values. Please explain rationales for using these parameters.

Authors' response: The reviewer's 6 and 7 comments/questions are essentially about the authors' rationales for using DTTv and DTTm in assessing the spatial and seasonal variability. The authors would like to address them both at the same time.

As stated in page 7252, line 11-12: "DTTm represents the intrinsic property of particles linked to sources, while DTTv accounts for the emission rate, dilution, etc., and characterizes exposure to the aerosol."

DTT activity per volume basis (DTTv) represents the "concentration" of DTT activity. The differences in DTTv give information on the different levels of exposure to DTT-active components, which is also very important to assess the health risks from toxic PM, but it doesn't provide much information on the sources. DTTm is the DTTv divided by $PM_{2.5}$ concentration, and thus is independent of how much $PM_{2.5}$ is present in the atmosphere. Therefore, the larger seasonal differences in DTTm might be attributed to the varying PM components that are DTT active in winter and summer and hence the sources might also be different. Based on this, the low spatial variability of DTTv and DTTm suggest dominant DTT sources are regional.

8. Section 3.2.4. Why DTTv (instead of DTTm) was used to find out correlation between DTT activity and PM2.5 mass concentrations? Based on the slope, it was concluded that the variations are due to varying PM chemical composition. It needs more discussion and logic behind such statement. Although, the authors tried to support the statement by the previous discussion on Anova/COD approach used for spatial/seasonal variability

Authors' response: As stated in the responses to reviewer's 6 and 7 comments, DTTm equals DTTv divided by PM concentration. The correlation of DTTm and PM2.5 mass concentration would not provide any physical meaning in interpreting the data. The slope of DTTv vs PM2.5 is equivalent to DTTm, thus the different slopes suggests different DTT-active PM components.

The following sentence is added to page 7258 line 24 to explain our logic as suggested: "The spatiotemporal variability of DTTv can be used to assess the exposure to total water-soluble DTT-active

species in different seasons and sites. And since DTTm is represented by DTTv divided by PM2.5 mass concentration, which is the intrinsic property of aerosol independent of PM2.5 mass, the spatiotemporal variability of DTTm provides information on the variation of DTT-active chemical components."

Bein, K. J., Wexler, A. S. A high-efficiency, low-bias method for extracting particulate matter from filter and impactor substrate, Atmospheric Environment, 90, 87-95, 2014.

Psichoudaki, M. and Pandis, S.N., Atmospheric Aerosol Water-Soluble Organic Carbon Measurement: A Theoretical Analysis, Environ. Sci. Technol., 47 (17), pp 9791–9798 DOI: 10.1021/es402270y, 2013.