

1 We would like to thank the referees for all the comments. The authors' responses are listed as
2 follows with corresponding changes made in the manuscript.

3
4
5 Anonymous Referee #1

6
7 In this study, the authors assessed the water-soluble oxidative potential of PM2.5 collected
8 in Southeastern U.S. based on both DTT and AA assays, and compared the results of two assays
9 in the view of their association with chemical components, sources and emergency department
10 (ED) visits. In my opinion, this is an important and careful study with large database, providing
11 essential information on the origin and potential health outcome of the water-soluble oxidative
12 potentials of PM2.5. In addition, the result could help future studies to better interpret the data
13 based on those assays. However, there are several issues that should be addressed in the
14 manuscript. A major concern is on the oxidative potential of PM2.5. Actually I'm afraid that
15 oxidative potential from water-soluble components is far from enough for the evaluation of
16 PM2.5's toxicity or health effects, given that a series of studies have suggested that some
17 hydrophobic components (e.g. represented by PAHs) from vehicles are the major toxic
18 components on human health (Delfino et al., Environ Health Perspect 2010, 118: 756-762; etc.).
19 Why not measure the oxidative potential of hydrophobic components if the authors wanted to
20 link the oxidative potential of PM2.5 with some health outcomes?

21
22 **Authors' response:** This paper does focus only on the water-soluble PM components
23 contributing to oxidative potential, and doesn't include the water-insoluble fraction. There is no
24 doubt that water-insoluble components also have potential adverse health impacts. There is a
25 logical reason to look separately at soluble vs insoluble components, however, since they are
26 likely to have differing physiological effects once deposited onto the aqueous environment of the
27 air-lung interface. Although we decided to focus solely on soluble species, we are not implying
28 that insoluble species are un-important, it is just not the focus of this work. Besides, there is no
29 standard protocol so far for measuring the water-insoluble ROS activities.

30
31 **Changes in manuscript:** Added to the Introduction section "Although in-soluble components
32 are important, since there is no current standard protocols for measuring the water-insoluble
33 oxidative potential, we focus solely on the water-soluble AA and DTT activities."

34
35 The following are some specific comments:

- 36 1. Page 30615, section 2.2.1: Could sonication for half an hour in the water phase generate
37 OH radical, which could result in great oxidative potential?

38
39 **Authors' response:** There is some concern on the alteration of the sample due to OH
40 formation during sonication [Miljevic et al., 2014]. We have conducted a limited number of
41 experiment on the effect of sonication on the measured oxidative potential by comparing
42 water-soluble DTT activity from the same extracts after sonication vs shaking for 3hrs. Our
43 findings suggest little difference between the two. Therefore, although sonication may
44 generate OH radicals, it doesn't seem to have significant effects on our DTT assay results. A
45 manuscript on the results is in preparation.

47 **Changes in manuscript:** page 30617 line 14, added “Although OH may form during
48 sonication (Miljevic et al., 2014), it has little effect on our ROS measurement since we compared
49 the water-soluble ROS activities from the same sample that had been extracted by shaking for 3
50 hours vs sonication and found no significant differences, average ratio is 1.08 ± 0.20 , $n = 7$.”
51

- 52
53 2. Page 30625, line 15: The spatio-temporal analysis could not draw the conclusion that the
54 oxidative potential is influenced by different components from different sources, because
55 there are no evidences in this part showed the similar trend for chemical components and
56 sources.

57 **Authors’ response:** We have removed the reference to chemical components.
58

59 **Changes in manuscript:** Page 30625 line 15: changed “These results indicate that water-
60 soluble DTT and AA activities of PM_{2.5} were influenced by different aerosol components
61 from differing sources that varied with season.” to “These results indicate that there are
62 differences in the sources for water-soluble AAv and DTTv, with traffic emissions
63 apparently a more significant source for AAv. Correlation analysis with specific aerosol
64 components provides further insights.”
65

- 66 3. Page 30626, line1: r_2 or r ? In case of r_2 , it’s better to convert it into r , since r is used
67 throughout the manuscript.
68

69 **Changes in manuscript:** page 30626 line 1: changed “ $r_2 > 0.5$ ” to “ $r > 0.7$ ”.
70

- 71 4. Page 30626, line 25: Since Pearson’s r is used. Please provide information on the
72 normality of the data and on whether data was log-transformed.
73

74 **Authors’ response:** As is found for almost all atmospheric constituents, their frequency
75 distributions are log-normally distributed. However, the correlation analyses were performed
76 using measured concentrations rather than log-transformed concentrations as this is a
77 straightforward interpretation method for these data. Since interpretation of the correlation
78 analysis results is similar whether done on a linear or log basis, we prefer to keep the former
79 here.
80

81 **Changes in manuscript:** No changes.
82

- 83
84 5. Page 30630, section 3.2.2: It is better to provide detailed data of the risk ratio (together
85 with 95% CI) in the text instead of in Fig. 4 only.
86

87 **Authors’ response:** The risk ratios for AA for asthma/wheeze and congestive heart failure
88 are added to the text. The detailed data for other health outcomes are provided as
89 supplemental material.
90

91 **Changes in manuscript:** Page 30630, line 13, changed “For asthma/wheeze and congestive
92 heart failure, the risk ratios for an increase of an interquartile range for DTT estimated from

93 the model (Eq. 6) were above 1 (Fig. 4), and the 95% confidence intervals exclude 1,
94 indicating a statistically significant positive association between estimated DTT and the ED
95 visits for these health outcomes.” to “For asthma/wheeze and congestive heart failure,
96 although the risk ratios for an increase of an interquartile range for AA_v^e were above 1 [1.005
97 and 1.003 for Asthma/wheeze and CHF, respectively, Fig. 5(a)], the 95 % confidence
98 intervals crossed 1 (0.994-1.015 and 0.986-1.020 for Asthma/wheeze and congestive heart
99 failure, respectively), indicating a non-statistically significant association between AA_v^e and
100 the ED visits for these health outcomes.”

- 101
102
103
104 6. Page 30631, line 3: The authors claimed that the epidemiology analysis “support aerosol
105 particle oxidative potential as a mechanism contributing to these PM-induced adverse
106 health effects”, which I think might not be true. A more robust association doesn’t
107 necessarily mean a possible mechanism. For example, sulfate is considered as “benign”
108 (page 30612, line 13), but actually sulfate is strongly associated with adverse health
109 effects in epidemiology studies, probably because sulfate was co-emitted with toxic
110 pollutants (Grahame, EHP, 2012). Personally, I think the association of DTT could also
111 possibly be attributed to co-emission with toxic pollutants, especially given that (1). DTT
112 is sensitive to organic species; (2). There is a lack of toxicology studies showing the
113 health effects of oxidative potential; (3). No significant association was observed for AA.
114 Although the authors attributed it to the different uncertainties (page 30630, line 19), it is
115 not convincing since r values of 0.60 and 0.68 are not that different.

116
117 **Authors’ response:**

118
119 First, we state that it “supports”, not that it proves OP is a mechanism. We would like to
120 address the reviewer’s three comments one by one:

- 121
122 1) DTT is sensitive to organic species
123 DTT assay is sensitive to organic species doesn’t prove that OP is not a plausible
124 mechanistic linkage. We’ve identified components of the organic species that are DTT
125 active [Verma *et al.*, 2015a; Verma *et al.*, 2015b]. For example, HULIS (humic-like
126 substances) in biomass burning or more-oxidized OA are mostly linked to DTT while
127 biogenic secondary organics basically have no DTT activity.
- 128
129 2) There is a lack of toxicology studies showing the health effects of oxidative potential
130 This is not true. There are numbers of studies have associated OP to PM toxicity, as we
131 have cited in the introduction section (see page 30612 line 27).
- 132
133 3) No significant association was observed for AA. Although the authors attributed it to the
134 different uncertainties (page 30630, line 19), it is not convincing since r values of 0.60
135 and 0.68 are not that different
136 We have rearranged the text to clarify the differences in health analysis between DTT and
137 AA are possibly due to the narrower selectivity of AA than DTT assay, rather than the
138 uncertainties in the models, which is fairly similar (ie, r values 0.60 vs 0.68).

139
140 In summary, although a positive association does not mean a causation, the association of
141 DTT with health effects is supported by a rational physiological mechanism (the DTT assay
142 measures the OP of particles, which represents the ability of PM components to generate
143 ROS and ROS can induce oxidative stress *in vivo*. It is also supported by our various findings
144 that DTT is sensitive to specific classes of organic species and metals that could lead to
145 health effects.

146
147 **Changes in manuscript:**

148
149 Page 30630 line 18: changed “Similar differences were found for estimates based...” to “The
150 same results were found for estimates based on the two other regressions [(see Figure 5(b)
151 and (c))], suggesting that the null relationship of AA_v^e and positive association of DTT_v^e with
152 these health outcomes are to some extent robust, despite the high uncertainties from the back-
153 cast models. A possible cause for the differences in AA_v^e and DTT_v^e health associations is the
154 more narrow selectivity of the AA assay to specific aerosol components (i.e., mostly
155 sensitive to Cu). The AA assay may not capture the overall oxidative potential of all the
156 various PM components as well as the DTT assay.”

157
158
159 Referee # 2 Cort Anastasio

160
161 Introduction.

162 Fang and co-authors describe results from two different measurements of oxidative potential –
163 DTT (dithiothreitol) and AA (ascorbic acid) – for ambient particles collected in the southeast
164 U.S. as part of the SCAPE center. The DTT and AA data sets are analyzed in three different
165 ways: (1) linear regressions between oxidative potential and various chemicals in an attempt to
166 identify the responsible chemical species; (2) positive matrix factorization (PMF) and chemical
167 mass balance (CMB) modeling to identify important sources of ROS-generating PM; and (3)
168 epidemiological modeling on approximately a decadelong time series of estimated DTT and AA
169 results to assess if either measure of oxidative potential is associated with health effects.

170 **Authors’ response:** We also investigated seasonal trends and spatial distributions.

171 The AA results are novel and, though not fully explored, form the core of a good manuscript.
172 With some additional pieces, this could be a very nice piece of work. On the other hand, the DTT
173 results have all been presented previously and there is nothing that warrants spending half the
174 manuscript on these past results. There are some new (and better) ways in which the DTT data
175 could be treated; if this is done, it could be an important contribution to our understanding of
176 DTT and would significantly improve the manuscript.

177 **Authors’ response:** First, we stress that this is a comparison paper. Our aim is to provide a
178 clear contrast between the AA vs DTT assays, rather than simply discuss AA results and
179 leaving it the reader to go back and forth between multiple papers. Admittedly, the AA
180 results do not show strong linkages to health (ie, a null result), but this is an important result.
181 However, the real strength of this paper is to provide a direct side-by-side comparison

182 between AA and DTT, which were both analyzed on the same filters. That is, we go through
183 a whole series of comparisons: spatial, seasonal, correlations to PM components, including
184 PM_{2.5} mass (important since it has been associated with adverse health effects in many
185 studies and is regulated), source apportionment, and then health effects. We have modified
186 the paper, including changing the title and figures, to emphasize the AA results. However, we
187 maintain the side-by-side comparison as we view this as a highly unique and powerful result.
188 In fact, this contrast largely addresses Major point 2 below, addressing the issue that DTT
189 may be only responding to a few metals and that this reviewer's analytical approach to assess
190 this assertion should be performed on this data set, as part of this paper.

191

192 **Changes in manuscript:**

- 193 1. We added the following in the introduction section to better illustrate our purpose, page
194 30614 line 21, added to the end "Throughout, we compare the AA results to our
195 previously published DTT findings (Bates et al., 2015, Fang et al., 2015b, Verma et al.,
196 2014) to provide a clear contrast between these two commonly utilized assays to assess
197 aerosol oxidative potential and possible associations with health endpoints."
- 198 2. Changed to the title to "Oxidative Potential of Ambient Water-Soluble PM_{2.5} in the
199 Southeastern United States: Contrasts in Sources and Health Associations between
200 Ascorbic Acid (AA) and Dithiothreitol (DTT) Assays".
- 201
- 202 3. Moved Figure S2 (Protocol schematics for conducting Ascorbic Acid assay) to the main
203 text.
- 204
- 205 4. Modified Figure 4 by putting AA health results first. To emphasize the robustness of epi
206 results from different models, Figure S7 (health results from the other two models) were
207 moved to the main text and combined with Figure 4.
- 208
- 209 5. Changes were made throughout the manuscript to increase the discussions of AA results
210 (see the complete manuscript with marked changes).
- 211

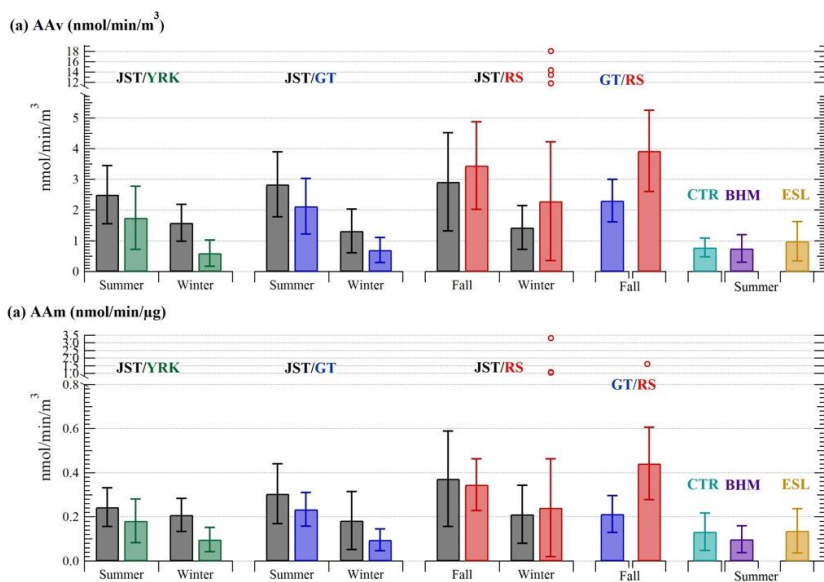
212 Major Points.

213 The DTT figures in the manuscript have all been shown (and discussed) previously: Figure 1b,
214 the DTT results in Figure 2, and Figures 3c and c are all from Verma et al. (2014), while the
215 DTT data in Figure 4 is from Bates et al. (2014). This previously published work represents
216 approximately half of the data in the manuscript. I appreciate that the authors want to compare
217 their new AA results with their old DTT results, but giving the two sets of data equal weight in
218 the manuscript takes away from the ascorbate findings. It also makes for a repetitious experience
219 for readers of Verma et al. (2014). My recommendation is to minimize the presentation of the
220 previous DTT figures and the discussion of the DTT results. The comparison of the DTT and AA
221 results is useful but could be done with a brief text discussion after each AA figure. Beyond
222 comparisons with AA, if the authors want to present significant amounts of DTT results in the
223 manuscript, they should be new; see the point #2 below for some suggestions on this.

224 The authors should more thoroughly present and discuss the AA results, as their treatment in the
225 manuscript is often weak. For example, the authors measured hundreds of samples, but only 17

226 monthly averages are presented. Is there anything interesting to show from the time series data?
 227 Is there anything interesting in the mass-normalized data? What do correlation plots of DTT and
 228 AA rates at the various sites show? What is the average value of the (DTT rate) / (AA rate) for
 229 each site/season; does this ratio say anything useful?

230 **Authors' response:** We have increased the discussions of AA, focusing the paper more on the
 231 AA results. As for the specific suggestions: Time series data essentially shows the same spatial
 232 and seasonal results as the average monthly data. We plotted the mass-normalized AA activity
 233 (in units of nmol/min/μg), see the graph below; which does not add significant new insights
 234 beyond what is already presented. DTT vs AA plots are very scattered and the ratio of DTT/AA
 235 is not very useful (this lack of correlation is important and discussed in the paper). Therefore, we
 236 don't find any of the above suggested analyses worthy of more detailed discussions than what is
 237 already presented in this paper.



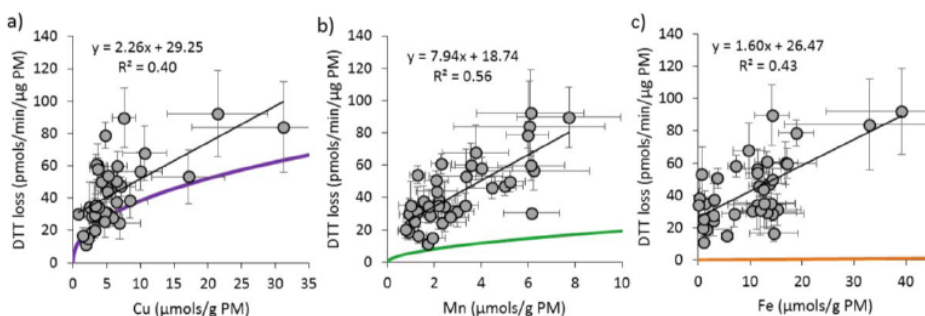
238

239

240 The authors have used linear regressions to assess the significance of metals and other
 241 components in the two assays. For DTT this analysis (e.g., Figure 2) is inappropriate because (1)
 242 two of the major contributors (Cu and Mn) have non-linear responses and (2) many of the
 243 components are correlated with each other. I ranted to the authors about this in my comments to
 244 Verma et al. (2014); see <http://www.atmos-chem-phys-discuss.net/14/19625/2014/acpd-14-19625-2014-discussion.html>. Since these past comments were largely ignored, allow me rant
 245 again, both for the purposes of the current manuscript and more broadly as a statement to
 246 DTT/ROS users.
 247

248 We respectfully disagree with the comments that our analysis is inappropriate and that we did not
249 already address them in a previous review (more on this below).

250 As an alternative to linear regressions we developed a mechanistic technique to quantify the
251 contributions of chemical species to the measured DTT (or other ROS) rate. This involves
252 measuring (1) concentration-response curves for each species (e.g., the rate of DTT loss as a
253 function of copper concentration), (2) concentrations of Cu and Mn in each sample, and (3) the
254 DTT rate of loss in each sample. We recently compared results from linear regressions and the
255 mechanistic approach for a set of samples from Fresno, California (Charrier et al., 2015). Our
256 mechanistic approach revealed that Cu, Mn, and unknown (likely organic) species account for an
257 average of approximately 50%, 20%, and 30%, respectively, of the measured rates of DTT loss
258 in these samples. These percentages are approximately

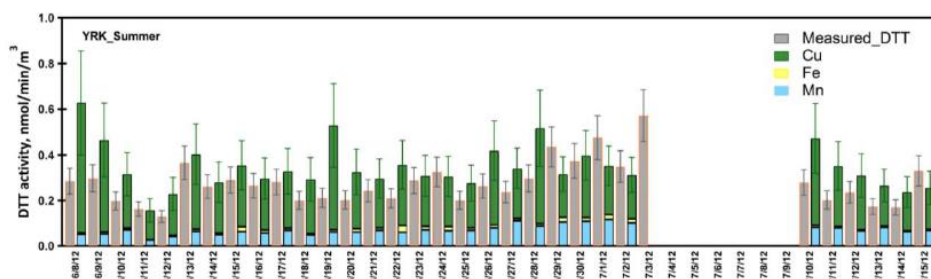


259 **Figure A.** Correlations between the measured rate of DTT loss and the concentrations of Cu, Mn, and Fe in samples from Fresno, California. Black lines represent the linear regressions. Each colored line represents the calculated rate of DTT loss from the metal based on our measured concentration-response curves (Charrier and Anastasio, 2012) and the typical PM mass loading of $10 \mu\text{g mL}^{-1}$ that was used in the assay in this study. Reproduced from Figure S8 of Charrier et al. (2015).

260 shown by the colored lines in Figure A. In contrast, the corresponding linear regressions for Cu,
261 Mn, and Fe show the weakest correlation for Cu ($R^2 = 0.40$) and the strongest for Mn ($R^2 = 0.56$).
262 The Fe correlation ($R^2 = 0.43$) is as strong as the copper correlation (and has a similar slope),
263 despite the fact that copper accounts for half of DTT loss and Fe accounts for essentially none.
264 Clearly regressions cannot be trusted to identify the species responsible for DTT loss.

265 Why does this matter for the Fang et al. manuscript? Because the authors have the opportunity to
266 use the mechanistic approach to better assess the contributions of Cu and Mn in their samples. In
267 response to my first review of Verma et al. (2014), the authors calculated the contributions of
268 these metals for their DTT rates with the mechanistic approach. These figures show that Cu
269 generally makes a major contribution to the SCAPE DTT; unfortunately, the figures can only be
270 found in the authors' second response to my comments (<http://www.atmos-chem-phys-discuss.net/14/19625/2014/acpd-14-19625-2014-AR2.pdf>) as they were not included in the final
271 version of Verma et al. (2014). In a subsequent paper, Verma et al. (2015), they assessed the
272 contributions of transition metals towards DTT but did so using linear regressions; these
273 correlations suggest that Cu and Mn are each important in only 3 of the 7 SCAPE sample sets
274 examined. In contrast, the mechanistic approach results show that Cu and Mn generally dominate
275 the DTT response at every site/season, although there are some problematic samples. As an
276

277 example, consider the YRK-June DTT data from Verma et al. (2015): linear regressions give R
 278 values of 0.64, 0.53, and 0.11 for Mn, Fe, and Cu, respectively. Since its regression fell below
 279 the R threshold, Cu was considered insignificant in these samples: the authors concluded that Mn
 280 and organics each accounted for approximately half of the DTT response, while Cu did not
 281 contribute. In contrast, the mechanistic approach for YRK-June (Figure B) shows that Cu
 282 generally dominates the DTT response, Mn is significant, and unknown components sometimes
 283 contribute. The measured and calculated rates for each sample in Figure B would likely agree
 284 better if the authors measured concentration-response curves on their automated system rather
 285 than used results from the manual runs in Charrier and Anastasio (2012). I encourage the authors
 286 to pursue this for the revised manuscript.

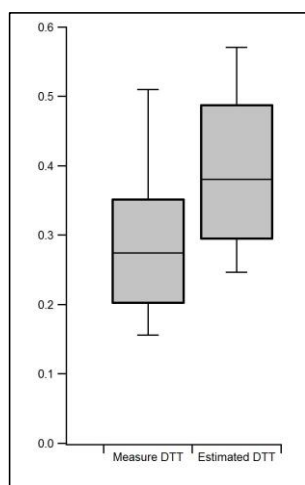


287 **Figure B.** Mechanistic assessment of the transition metal contributions to the DTT response in the YRK-June (and
 288 July) samples. From the Verma et al. response (29 October 2014) to my comments to Verma et al. (2014); see
 289 <http://www.atmos-chem-phys-discuss.net/14/19625/2014/acpd-14-19625-2014-AR2.pdf>

288 It is an open question whether Cu and Mn also have non-linear responses in the AA assay. If
 289 they do, then the linear regression assessment of which species contribute to the AA result in the
 290 manuscript might have problems. Since it would be a simple matter to measure the
 291 concentration-response curves for these metals in the authors' automated system, I recommend
 292 that they make these measurements. The authors should also use these curves to assess transition
 293 metal contributions to AA using a mechanistic approach and compare it to the regression results.

294
 295 **Authors' response:** We have not performed the requested analysis because we do not feel it is
 296 informative, i.e., it does not definitively show what components are contributing to the observed
 297 activity), largely because the uncertainties in the reviewers mechanistic method are very large,
 298 such that it is difficult to infer definitive evidence for what species are contribution to the
 299 observed activity. The fact that this method indicates that DTT is controlled by Cu and Mn,
 300 whereas we have strong evidence that this is not the case tends to support this conclusion.
 301 Although we have addressed this before in a previous paper, we will summarize our arguments
 302 again since they are stronger, in part due to the findings presented in this paper, that DTT activity
 303 is not simply due to Cu and Mn. First, we summarize Figure B above by combining all the data.
 304 The result is shown below as a bar/whisker plot. The summation method proposed by this
 305 reviewer, based on our measurements of water soluble Mn and Cu, significantly over-predicts
 306 DTT relative to the observed DTT, and possibly more importantly the difference is highly
 307 variable. This is also clearly demonstrated by the r^2 between measured and estimated DTT of

308 0.22 (ie, the summation method can only explain 22% of the observed DTT variability). As we
309 have noted before, the results appear inconclusive and the method has little predictive capability.
310 (A similar conclusion is reached if one simply considers the uncertainty in the method, when all
311 uncertainties are propagated). Instead of basing an analysis on laboratory experiments using pure
312 compounds in isolation from the chemically complex mixtures found in ambient aerosols, we
313 prefer to be guided by ambient data based on multiple comparisons, not all of which are
314 correlation analyses or limited by possible nonlinear responses, ie, spatial and seasonal
315 distributions, and mechanistic approaches, see below. Overall, our comparisons and analysis
316 provides a consistent conclusion that DTT activity in our study was not driven solely by Mn and
317 Cu. .



318 It seems, however, the main point of our paper has
319 largely been missed by this reviewer. That is, for all
320 the various comparisons outlined here, there are
321 distinct contrasts between the DTT and AA assays,
322 and the two assays are not themselves well
323 correlated. If DTT was only responsive to Cu and
324 Mn, (like AA), the stark contrasts between DTT and
325 AA in the ambient data outlined here would not be
326 expected. Again, we feel this demonstrates the
327 strength of our papers format of a side-by-side
328 comparison between these assays.

329 In further support of our approach, next we
330 summarize the evidence that DTT is driven by more
331 than Cu and Mn.

332 1) The major emission source found to be most
333 influential from our two different source
334 apportionment models (PMF and CMB-E) is
335 biomass burning (Figure 3). It is known that

336 biomass burning is not a large source for Cu and Mn, and indeed our study shows that only 16%
337 of the Mn and 0% of Cu is associated with this source [Fang *et al.*, 2015a]. Biomass burning,
338 however, is a known large source of OA, specifically HULIS, which we (and others) have shown
339 is related to DTT activity and which contains compounds known to be DTT active (eg.,
340 quinones, , hydroxyquinones, etc.). Furthermore, in this paper we show AA is mostly associated
341 with these metals and biomass burning is not a source for AA. The seasonal differences in DTT
342 and AA, also reported in this paper, provide further support; where DTT activities are higher in
343 winter at some sites, AA activities were not (AA actually has the opposite trend, being higher in
344 summer), consistent with known trends in biomass burning emissions (ie, higher in winter) and
345 confirmed by our tracers. Likewise, AA source apportionment indicated it was more associated
346 with traffic sources, which is supported by the spatial distributions; AA was observed to be
347 higher at urban vs rural sites, and amongst the urban sites higher at sites closer to traffic
348 emissions. DTT does not show similar spatial trends. Thus, although the source apportionment
349 may be affected by co-variabilities and non-linear responses, the season and spatial trends are not,
350 and the two independent analyses are consistent.

351 2) Another important point discussed in this paper is that DTT was highly correlated with PM_{2.5}
352 mass ($r=0.49-0.88$ [Fang *et al.*, 2015c]). Mn and Cu do not correlate with PM_{2.5} mass at most
353 of times, ($r=-0.18-0.66$, two exceptions are at GT and RS winter time, $r>0.75$ for Mn), which
354 is not surprising as they are small mass fractions of PM_{2.5}. If Cu and Mn were the main
355 drivers of DTT as well, one wouldn't expect to see the high correlations between DTT and
356 PM mass. This is noteworthy since it is consistent with our epi comparisons between DTT
357 and AA, and the many studies showing linkages between PM_{2.5} mass and health. DTT is
358 correlated with PM_{2.5} and DTT shows associations with some health endpoints, AA is not
359 correlated with PM_{2.5} mass and shows less association with health endpoints.

360 3) This reviewer prefers a mechanistic analysis versus correlations. We have also done this and
361 the results are consistent with the correlation analyses. A mechanistic approach that
362 quantified the contributions of OA vs metals to DTT has been presented [Verma *et al.*,
363 2015a]. In that study we passed the water-soluble extracts, in which both water-soluble DTT
364 activity and metals concentrations had been measured, through a C-18 column to separate
365 hydrophilic and hydrophobic components. Both the DTT activity and metals concentrations
366 were measured in the fraction that passed through the column (hydrophilic fraction). The
367 hydrophobic fraction can be determined by the difference between total and hydrophilic. We
368 found that hydrophobic DTT activity made up a large fraction of total water-soluble DTT
369 activity and Cu and Mn were mostly excluded from the hydrophobic fraction. These results
370 indicated that the DTT activity of the water-soluble hydrophobic fraction was not driven by
371 Cu/Mn, but another class of redox-active species, most probably organic compounds
372 associated with HULIS. From this analyses we reported that roughly 60% of the DTT
373 activity in that study was associated with the OA and 40% metals. As OA from biomass
374 burning is a major source for HULIS, this is consistent with the various source
375 apportionment and correlation analysis discussed above. Finally, we also did a mass balance
376 analysis, much like the reviewer has done, and found that including the OA (ie, hydrophobic
377 DTT activity) provided better closure.

378

379 4) As further proof that DTT is not solely driven by metals we have performed a traffic
380 tunnel/chamber study, which has shown high DTT activity for ambient aerosol containing no
381 metals. In yet-to-be-published work we performed experiments on sample air that was drawn
382 from a highly trafficked vehicle tunnel. Tunnel air could be analyzed directly or modified. In
383 one experiment, the sample air was filtered to remove all aerosols (which includes all
384 metals). The sample was then directed to a smog chamber and exposed to lights, resulting in
385 SOA from the VOCs in the tunnel. This SOA was highly DTT active, with levels comparable
386 to the hydrophobic fraction of the ambient aerosols observed in SCAPE.

387

388 In summary, although this reviewer's point is well taken, that correlation is not causation, the
389 totality of our uniquely large data set strongly point to the role of both OA and metals to the
390 DTT activity of water soluble components. It includes analyses that are not subject to the
391 limitations pointed out (correlation analysis and possible nonlinear responses). The unique
392 strength of our data is that it is large and includes long time series (ie, seasonal data), and data
393 from multiple sites (ie, spatial data). This is in contrast to the reviewer's work, from which they

394 conclude that DTT is mainly driven by Cu and Mn. We have also shown above that the analysis
395 proposed, does not provide a definitive indication that DT activity is driven solely by Mn and
396 Cu, for our data set. And finally, based on this paper comparing DTT and AA, the Verma et al.,
397 (2015) paper and our tunnel data, we conclude that the approach used by this reviewer does not
398 provide an informative way to assess the contributions of aerosol species to the water-soluble
399 DTT activity of ambient particles measured in our study. Finally, some time back we attempted
400 the mechanistic approach, developing a calibration for AA response to pure Cu solutions made
401 from copper sulfate. Although only exploratory in nature, we found the calibration curve to be
402 somewhat unstable and the predicted AA, based on the curve and our measured water soluble
403 Cu, greatly under predicted the AA activity relative to observed (10% of observed). For all of
404 these reasons, we chose not to include this reviewer's mechanistic approach in the analyses of
405 our AA data set.

406

407 4. Backcast estimates of AA and DTT activities and the epidemiological analyses. A discussion
408 of the uncertainties in the backcast estimates of the AA response is needed. The uncertainties
409 must be very large, as illustrated for DTT in Figure 1 of Bates et al. (2015). The equivalent
410 figure for AA should be shown in the manuscript. As part of the discussion, how can the
411 backcast uncertainties of AA (and DTT) activity be enormous, but the 95% confidence intervals
412 around the RR data points in Figure 4 be quite small. Do the Figure 4 CIs include the full
413 backcast uncertainties? How are these propagated?

414 **Authors' response:** The CIs in the figure are for the epi results and do not include uncertainty in
415 the backcast DTT or AA. See comments to minor point 3 below. Our argument is that the AA
416 and DTT models do roughly equally well when compared to the ambient data (similar r). How
417 well they predict actual historical DTT and AA is not known, but we have no reason to believe
418 that AA is predicted much worse than DTT. In fact one might expect the opposite as DTT is
419 driven by more sources than AA. In any case, the point is that DTT shows health associations
420 and the AA does not. This is an important conclusion despite the high uncertainties with the
421 methods, and is consistent with the contrasts described throughout the paper. It again stresses the
422 value of the side-by-side comparison.

423

424 **Changes in manuscript:** No changes.

425

426 Since AA is dominated by Cu, and there are enormous uncertainties in the backcast estimates of
427 AA (and DTT) activity, it would be interesting to do the epidemiological modeling using
428 measured particulate Cu rather than the predicted AA response. This might show a significant
429 correlation with the health endpoints. Are there historical data in Atlanta that could be used for
430 this? If this epidemiological analysis could be done relatively easily, I encourage the authors to
431 include it in the current manuscript. If not, I hope to see it in a future manuscript.

432 **Authors' response:** We have considered this. Unfortunately, a sufficient water-soluble Cu data
433 does (ie. Sufficient number of data above LOD) not exist for the time period of the epi study.
434 Thus, running the epi with just water-soluble Cu would still require a backcast prediction.

435

436 **Changes in manuscript:** No change was made.

437

438 Minor Points.

439 1. “AA” is used to represent the volume-normalized rate of AA loss, but of course it’s also the
440 name of AA itself, which is confusing. Better to use something like “AA_v” for the rate,
441 analogous to the DTT nomenclature of Verma et al. (2015). There is the same issue for DTT in
442 the manuscript.

443 **Authors’ response:** Replaced DTT and AA activity with DTT_v and AA_v throughout the paper.
444 Also used DTT_v^e and AA_v^e for backcast estimates of DTT_v and AA_v.
445

446 2. p.30630. The authors should include the results for the other health outcomes (COPD,
447 pneumonia, IHD) in Table S4, as the comparison with asthma and CHF would be interesting.

448 **Authors’ response:** We agree and added the health outcomes of COPD, pneumonia, and IHD to
449 Table S4.

450

451 **Changes in manuscript:** added the data of health risk ratios of COPD, IHD, and pneumonia to
452 Table S4. In the main text, page 30630 line 12: changed “(results not presented)” to “(results
453 given in Table S4)”.

454

455 3. p. 30631. The authors conclude that “For the region investigated in this study, the DTT assay
456 was a more comprehensive multi-pollutant ROS (or oxidative potential) indicator than the AA
457 assay making DTT a potentially valuable parameter to include in future PM health-related
458 studies.” Given the very small differences in the RRs for DTT and AA, and the very large
459 uncertainties in their backcast estimates, this conclusion is far too strong.

460 **Authors’ response:** We disagree when one considers all aspects of the comparisons laid out in
461 this paper. Admittedly, the back-cast prediction of DTT and AA activities has large uncertainties,
462 but again it is the comparisons that are insightful; DTT shows statistically significant
463 associations (at 95% confidence level), AA does not. And this is true for all regression models
464 we used to backcast DTT and AA. We have now added these results to Figure 4 to emphasis the
465 point.

466

467 Additionally, previous studies has shown good correlation between DTT and biomarkers such as
468 hemeoxygenase expression in cells and exhaled nitric oxide fraction [Delfino et al., 2013; Li et
469 al., 2003]. We believe that DTT assay is a valid accellular assay for studying the ROS-generation
470 *in vivo* and would be valuable to include in future PM health-related studies.

471

472 **Changes in manuscript:** Page 30630 line 18, edited as “The same results were found for
473 estimates based on the two other regressions [(see Figure 5(b) and (c))], suggesting that the null
474 relationship of AA_v^e and positive association of DTT_v^e with these health outcomes are to some
475 extent robust, despite the high uncertainties from the back-cast models.”
476

477 4. Finally, I want to apologize to the authors for taking so long to complete this review.

478

479

480 Anonymous Referee #3

481

482 Summary

483 This manuscript uses a number of important methods to bridge the continuing gap between
484 detailed composition information and the association between PM and negative health effects.
485 The DTT and AA assays were run on PM extracted from a number of locations across two year
486 in the southeast United States. Correlations were made between the DTT and AA assays and
487 PMF and CMB modeling of sources and with ED admissions from long term epidemiological
488 data. On the whole I feel the paper has a lot of strong characteristics, particularly the new assay
489 methodologies and their connection with source modeling. I think the paper overall is fairly
490 strong and should be accepted with minor revisions.

491

492 Major Comments

493 One concern is that there is a lot of detail and information in the methods section, but that the
494 results and discussion section feels light on new results. As noted by another reviewer, a large
495 amount of the DTT data has been shown before in prior publications. Though comparisons with
496 prior data are good, it feels a little like a second bit at the apple for the DTT data. A revised
497 manuscript that shows more data from the AA results would be compelling, though perhaps due
498 to the strong Cu response there was concern that the AA results were not strong enough on their
499 own? I rarely suggest making a paper longer, but at 18 pages with only 4 of results and
500 discussion that could really be expanded and strengthened.

501

502 **Authors' response:** Since this work has combined field campaign, air quality modeling and
503 epidemiologic analysis, it involves extensive methodological descriptions. We have eliminated
504 the replicate method descriptions, now providing just references, and have moved them to the
505 supplemental materials. These include the method description of DTT assay from Fang et al.
506 (2015b) and water-soluble elements from Fang et al. (2015a).

507

508 We have also revised the paper, putting more emphasis on the AA results. Also see our 2nd
509 response to the 2nd reviewer's comments.

510

511 **Changes in manuscript:** Moved the method description of DTT assay (page 30615 section
512 2.2.1) and a large fraction of "water-soluble elements" (page 30620 section 2.3.2) to the
513 supplement and added text emphasizing the AA results.

514

515 The extensive literature citations are commendable and the introduction very nicely sets up the
516 need for this work. Along the lines of the previous comment, at times I felt like the new findings
517 from this study got lost in the sea of references to this group of authors previous papers,
518 particularly those from the last 2 years and Balachandran et al. 2012. A revised manuscript that

519 more clearly delineates the findings of this study from prior work will help readers understand
520 the findings of this work. Though the data is admittedly abundant and at times dense, the results
521 section feels like a list of numbers in a few paragraphs. Rewording to bring the science out from
522 behind these numbers would be helpful.

523 **Authors' response:** We have eliminated repetitive references to previous publications and have
524 reworded most sections to emphasize the results from AA.
525

526 In Figure 3 the amount of AA response from brake/tire wear being 44% is notable. I worry that
527 since as the authors note that these are mechanically generated particles that they are likely solid
528 and smaller than the 0.45 micron filter used in the methods. The still solid metals might only be
529 dissolved when nitric acid is introduced prior to the XRF analysis. If so this would seem to
530 suggest that this is not truly a water soluble, but rather an insoluble source that becomes soluble
531 during analysis. Overall the possibility of solid particles less than the 0.45 um filter should be
532 addressed in more detail. If this concern is plausible then some of the phrasing in the manuscript
533 (and potentially even the title) should be qualified since much of the AA response might be from
534 insoluble material.

535 **Authors' response:** If the particles are formed mechanically, they are not expected to be smaller
536 than 0.45 μm in significant quantities. It is established that mechanically generated aerosol are
537 mainly found in the coarse mode, but which can extend down to $\sim 1\mu\text{m}$ (eg, see Seinfeld and
538 Pandis). Thus, some fraction of these coarse particles will be included in PM_{2.5}, but very few
539 below 0.45 μm . (Note, we have measured the size distribution of the AA activity and there is
540 practically no contribution to the AA activity from particles below 0.45 μm). Thus, we expect
541 few solid particle to interfere with our measurement. Regarding the nitric acid: Nitric acid is
542 added to the extract after the extract is filtered, so it will not add to the water soluble metals
543 signal if there are insoluble particle in the extract (ie, particles below 0.45 μm).
544

545 **Changes in manuscript:** No changes.
546

547 AA is used as a surrogate for a more complex lung fluid and found to only really correlate with
548 Cu. Are the authors concerned that perhaps this more simplistic model fluid is not capturing
549 sensitivity to other species that perhaps a more complex simulated fluid might? A little bit of
550 further discussion on whether AA is really a great choice would be helpful, a otherwise it might
551 not be sensitive enough to mimic the responses in the lung that are observed from other types of
552 studies.

553 **Authors' response:** Yes, the AA assay used in our work (as stated in page 30617 line 14) is a
554 more simplistic model to a synthetic respiratory tract lining fluid model (RTLFL), which contains,
555 besides AA, urate and reduced glutathione (GSH). This ascorbate-only method has been used by
556 other studies [Ayres *et al.*, 2008; Mudway *et al.*, 2005] to provide an alternative high throughput
557 method to synthetic RTLFL. In their work, AA-only method was performed to characterize the
558 metal dependence of oxidative reactions, mimicking the nature of particles-antioxidant (i.e. AA)
559 interactions at the air lung interface.
560
561

562 **Changes in manuscript:** page 30617 line 14, changed “The method in this study was based on
563 an ascorbate-only model (Mudway et al., 2005; Ayres et al., 2008) that is a simplified approach
564 to a synthetic respiratory ...” To “The method in this study was based on an ascorbate-only
565 model (Mudway et al., 2005; Ayres et al., 2008) that is a simplified and alternative high
566 throughput approach to a synthetic respiratory...”
567

568 Minor Comments/Concerns

569 LWCC is defined on both 30617 and 30619

570 **Authors’ response:** deleted the 2nd expansion of LWCC.

571
572 **Changes in manuscript:** Page 30619 line 19: changed “Liquid Wave-guide Capillary Cell” to
573 “LWCC”.
574

575 700 nm was chosen as the background, but some things in the atmosphere can absorb at that
576 wavelength. Washenfelder et al. 2015 GRL showed episodic variation of absorption for the 4
577 wavelength of aerosol absorption they monitored (highest was 530 nm). Have the authors done
578 checks with full absorption spectra to determine if 700 nm is truly a safe choice for background?
579 This would be particularly important when biomass is playing such an important role in both
580 absorption and DTT assay activity.

581 **Authors’ response:** We developed the method used to measure Brown Carbon [*Hecobian et al.*,
582 2010] and made the measurements (ie, it is our data) and can say definitively that there is no
583 absorption at 700 nm from other aerosol components.

584
585 **Changes in manuscript:** No changes.

586
587 As a curiosity is there a reason that XRF was used and not ICP-MS?

588 **Authors’ response:** It was the method available and also easily adaptable to handle the large
589 number of filters we had to analyze. To send out the hundreds of samples for analysis by ICP-
590 MS would have lead to exorbitant costs (ie, over \$100/sample)

591
592 **Changes in manuscript:** No changes.
593

594 This might just be a lack of knowledge, but ammonium bisulfate is a class listed for one of the
595 models. How is that determined and separated from ammonium sulfate? Is that from
596 ISOROPPIA or some other thermodynamic model?

597 **Authors’ response:** The separation of ammonium sulfate and ammonium bisulfate is based on
598 the differences in their source profiles, which were determined from an ensemble average of
599 different CMB profiles and CMAQ results. The main distinguishing drivers in the source profiles
600 are the average mass ratio of ammonium/sulfate. A thermodynamic model (ie, ISORROPIA) is
601 not used.
602

603 **Changes in manuscript:** No changes.
604

605 Section 2.3.3 Though referencing the other work is important, I am a bit suspicious of using PM
606 mass concentrations estimated from the sum of chemical component from Hi-Vol samples. More
607 detail on how this has been justified (even one sentence), beyond just referencing citations
608 would appease that concern.

609 **Authors' response:** Since total PM_{2.5} measurement was not available for the RS and GT site, we
610 had to use the summation method. Added the statement below to clarify.

611 **Changes in manuscript:** Page 30620 line 20: changed "For the RS and GT sites, the PM mass
612 concentrations were estimated from the sum of chemical components analyzed on the same Hi-
613 Vol" to "For the RS and GT sites, since PM_{2.5} mass were not available, the PM mass
614 concentrations were estimated from the sum of chemical components analyzed on the same Hi-
615 Vol filters (Verma et al., 2014) (Details in the Supplement)."
616
617

618 Are the %'s (filter sampling, etc.) chosen for propagating uncertainty on page 30632 from a
619 reference or chosen arbitrarily? I was not clear from the text where they came from.

620 **Authors' response:** Our previous work [Fang et al., 2015b] has discussed in details the
621 uncertainties from the method, including analytical uncertainty (2-15%), calibration uncertainty
622 (1-6%) , and blank variability. We have found that the overall precisions from co-located
623 comparisons (1σ/slope) are 6-20%. From this, we estimated the specific uncertainties for filter
624 sampling and extraction to be 5%.
625

626 **Changes in manuscript:** Page 30621 line 16, changed "which were obtained by propagating the
627 uncertainties from filter sampling (5%), extraction (5%)" to "which were obtained by
628 propagating the uncertainties from filter sampling (assumed to be 5%), extraction (assumed to be
629 5%)".
630

631 Page 30630: "the model did not observed" should be "the model did not observe"

632 **Authors' response:** edited as suggested.
633

634 **Changes in manuscript:** page 30630 line 31: changed "the model did not observed" to "the
635 model did not observe".
636

637
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674

675

676 **Oxidative Potential of Ambient Water-Soluble PM_{2.5}**
677 **Measured by Dithiothreitol (DTT) and Ascorbic Acid (AA)**
678 **Assays in the Southeastern United States: Contrasts in**
679 **Sources and Health Associations between Ascorbic Acid**
680 **(AA) and Dithiothreitol (DTT) Assays**

681
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706

707 **Abstract**

708 The ability of certain components of particulate matter to induce oxidative stress through catalytic
709 generation of reactive oxygen species (ROS) *in vivo* may be one mechanism accounting for
710 observed linkages between ambient aerosols and adverse health outcomes. A variety of assays
711 have been used to measure this so-called aerosol oxidative potential. We developed a semi-
712 automated system to quantify oxidative potential of filter aqueous extracts utilizing the
713 dithiothreitol (DTT) assay and have recently developed a similar semi-automated system using the
714 ascorbic acid (AA) assay. Approximately 500 PM_{2.5} filter samples collected in contrasting
715 locations in the Southeastern US were analyzed for a host of aerosol species, along with AA and
716 DTT activities. Here we present a detailed contrast in findings from these two assays, using both
717 assays. We found that wWater-soluble DTT activity on a per air volume basis was more spatially
718 uniform than water-soluble AA activity. DTT-AA activity was higher in summer/fall winter than
719 in winter-summer/fall, with highest levels near highly trafficked highways, whereas AA-DTT
720 activity was higher in winter summer/fall—compared to summer/fall and more spatially
721 homogeneous winter, with highest levels near highly trafficked highways. AA activity was nearly
722 exclusively correlated with water-soluble Cu (r = 0.70-0.94 at most sites), whereas DTT activity
723 was correlated with organic and metal species, whereas AA activity was correlated with water-
724 soluble metals (especially water-soluble Cu, r = 0.70-0.91 at most sites). Source apportionment
725 models, Positive Matrix Factorization (PMF) and a Chemical Mass Balance Method with
726 ensemble-averaged source impact profiles (CMB-E), suggest a strong contribution from secondary
727 processes (e.g., organic aerosol oxidation or metal mobilization by formation of an aqueous
728 particle with secondary acids) and traffic emissions to both AA DTT and DTT AA activities in
729 urban Atlanta. Biomass burning was a large source for DTT activity, but insignificant for AA. AA
730 activity DTT activity was well not correlated with PM_{2.5} mass (r = 0.49-0.86 across sites/seasons),
731 while DTT activity AA activity did not co-varied strongly with mass (r = 0.49-0.86 across
732 sites/seasons). A Various linear models was were developed to estimate AA DTT and DTT AA
733 activities for the central Atlanta Jefferson Street site, based on the CMB-E sources that are
734 statistically significant with positive coefficients. The models was were then used to estimate daily
735 oxidative potential at this site over the period 1998-2009 period. Time-series epidemiological
736 analyses were conducted to assess daily emergency department (ED) visits data for the five-county
737 Atlanta metropolitan area based on the estimated 10-year backcast oxidative potential. Results
738 suggest that estimated AA activity was not statistically associated with any tested health outcomes,
739 while DTT activity was associated with ED visits for both asthma/wheeze and congestive heart
740 failure, while AA activity was not linked to any health outcomes. The findings point to the
741 importance of both organic components and transition metals from biomass burning and mobile
742 sources to adverse health outcomes in this region.

743

744 **Key Words:** water-soluble DTT activity; water-soluble AA activity; oxidative potential;
745 **source apportionment; health associations; cardiovascular; respiratory**

746

747 1. Introduction

748 Studies have linked exposure to fine particulate matter (PM_{2.5}) with increased respiratory
749 (Harkema et al., 2004; Aust, 2002; Schaumann et al., 2004) and cardiovascular (Pope et al.,
750 2004; Samet et al., 2000) diseases. PM_{2.5} consists of a wide range of chemical components of
751 potentially varying toxicity, implying that PM_{2.5} is not an ideal air quality metric for assessing
752 health impacts. For example, components such as ammonium, sulfate, nitrate, chloride, and some
753 chemical fraction of mineral dust, may be more benign than transition metals (Gasser et al.,
754 2009; Kodavanti et al., 2005; Akhtar et al., 2010), black carbon (or elemental carbon and
755 associated species) (Kleinman et al., 2007; Brunekreef et al., 1997), polycyclic aromatic
756 hydrocarbons (PAHs) (Lundstedt et al., 2007; Burchiel et al., 2005), and other specific organics
757 species (Nel et al., 2001). Although a small mass fraction of PM_{2.5}, these components ~~may could~~
758 play a disproportionately large role in the overall adverse health effects of PM_{2.5}. ~~In contrast,~~
759 ~~Harrison et al. (2000) notes that the consistency of epidemiological associations between PM_{2.5}~~
760 ~~mass and health effects across regions of widely varying aerosol sources implies that individual~~
761 ~~chemical components comprising small mass fractions of PM_{2.5} cannot be responsible for~~
762 ~~observed PM_{2.5}-health associations.~~ A comprehensive set of mechanisms explaining the observed
763 linkage between PM_{2.5} mass and adverse health effects has not been established, but it has been
764 hypothesized that one possible contributing ~~mechanism-physiological route~~ is a particle's ability
765 to induce oxidative stress via catalytic generation of reactive oxygen species (ROS), *in vivo*. A
766 number of studies have associated particle oxidative capacity with PM toxicity (Donaldson et al.,
767 2005; Nel, 2005; Shi et al., 2003; Zielinski et al., 1999), but without available large databases of

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768 ambient aerosol ROS, large population-based epidemiologic studies of PM_{2.5} oxidative potential
769 have not been possible.

770 A number of different assays have been developed to quantify the oxidative potential of PM
771 samples (Zomer et al., 2011; Mudway et al., 2011; Ayres et al., 2008; Jung et al., 2006; Cho et
772 al., 2005; Mudway et al., 2005; Venkatachari et al., 2005). Two commonly used approaches are
773 the dithiothreitol (DTT) assay (Cho et al., 2005) and here describe its adaptation to the ascorbic
774 acid (AA) assay (Ayres et al., 2008; Mudway et al., 2005). It may be expected that these two
775 different assays respond to different aerosol components and are linked to different health
776 endpoints. The AA assay has been shown to be most sensitive to transition metals (Janssen et al.,
777 2014; Strak et al., 2012; DiStefano et al., 2009; Künzli et al., 2006) but quinone compounds may
778 react with AA as well (Roginsky et al., 1999). For the DTT assay, identified DTT-active PM
779 components are organic species, including water-soluble organic carbon (WSOC) (Verma et al.,
780 2009; Cho et al., 2005), or of increasing specificity, HUMic-Like Substances (HULIS) (Verma et
781 al., 2012; Lin and Yu, 2011), and quinones (Chung et al., 2006; Kumagai et al., 2002) (a
782 component of HULIS). Other studies, however, have emphasized the role of transition metals,
783 such as Cu and Mn (Vejerano et al., 2015; Charrier and Anastasio, 2012). Some differences
784 between studies may arise due to differing source characteristics of the specific regions studied.
785 Both assays involve incubating the anti-oxidant (DTT or AA) with filter aqueous extracts of
786 PM_{2.5} at a controlled temperature (37 °C) and pH (7.4), and measuring the depletion of the
787 antioxidant over time, typically detected as a decrease in absorbance ~~light absorption~~ at a certain
788 wavelengths of (412 and 265 nm for DTT and AA, respectively). The antioxidant loss rate is
789 interpreted as a measure of the ability of aerosol redox-active species to catalytically transfer
790 electrons from DTT or AA to oxygen (O₂). DTT can be considered a chemical surrogate to

Field Code Changed

791 cellular reductants, such as NADH or NADPH, which reduces O₂ to superoxide anion (O₂^{·-}) and
792 induces oxidative stress (Kumagai et al., 2002). Unlike DTT, AA is a physiological antioxidant
793 in lung lining fluid, which prevents the oxidation of lipids and proteins (Valko et al., 2005).
794 Asthmatic patients have markedly decreased concentration of AA in lung lining fluid compared
795 to healthy control subjects (Kelly et al., 1999). Therefore, the *in vitro* oxidation of these two
796 antioxidants by PM might represent the interaction of PM with biological antioxidants *within the*
797 *body in vivo* leading to the induction of oxidative stress and ultimately adverse health effects.
798 Among the various available methods for measuring oxidative potential, these two assays are
799 relatively straightforward and reproducible, allowing high throughput routine measurements and
800 the generation of large data sets for exploring links between aerosol components and health
801 through epidemiology, or also as an initial screening step for identifying different redox
802 components for more detailed cell or animal studies (Ayres et al., 2008).

803 We recently developed a semi-automated system (Fang et al., 2015b) to measure DTT activity
804 and here describe its adaption to the AA assay. Utilizing our automated analytical system, we
805 measured the water-soluble oxidative potential of over 500 filter samples collected as part of the
806 Southeastern Center for Air Pollution & Epidemiology (SCAPE) study. Although in-soluble
807 components are important, since there is no current standard protocol for measuring the water-
808 insoluble oxidative potential, we focus solely on the water-soluble AA and DTT activities. We
809 evaluate and compare these two assays in order to identify specific aerosol components the AA
810 assay is responsive to. We perform a source apportionment analysis and assess these results
811 through observed AA activity seasonal and spatial variability. AA source profiles are used to
812 generate a model that estimates AA activities, which is then used to backcast AA levels over the
813 past 10 years for use in a time-series epidemiological analysis in the Atlanta metropolitan area.

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814 Throughout, we compare the AA results to our previously published DTT findings (Bates et al.,
815 2015, Fang et al., 2015b, Verma et al., 2014) to provide a contrast between these two commonly
816 utilized assays to assess aerosol water-soluble oxidative potential and possible associations with
817 health endpoints.

818
819 ~~quantify oxidative potential of PM samples utilizing the dithiothreitol (DTT) assay (Cho et al.,~~
820 ~~2005) and here describe its adaptation to the ascorbic acid (AA) assay (Ayres et al., 2008;~~
821 ~~Mudway et al., 2005). Among the various available methods for measuring oxidative potential,~~
822 ~~these two assays are relatively straightforward and reproducible, allowing high throughput~~
823 ~~routine measurements and the generation of large data sets for exploring links between aerosol~~
824 ~~components and health through epidemiology, or also as an initial screening step for identifying~~
825 ~~different redox components for more detailed cell or animal studies (Ayres et al., 2008). Janssen~~
826 ~~et al. (2015) investigated the two assays in a panel study (n=31) and found significant~~
827 ~~associations between both assays and nasal and airway inflammation. Both assays involve~~
828 ~~incubating the anti-oxidant (DTT or AA) with filter aqueous extracts of PM_{2.5} at a controlled~~
829 ~~temperature (37 °C) and pH (7.4), and measuring the depletion of the antioxidant over time,~~
830 ~~typically detected as a decrease in absorbance at wavelengths of 412 and 265 nm for DTT and~~
831 ~~AA, respectively. The antioxidant loss rate is interpreted as a measure of the ability of aerosol~~
832 ~~redox active species to catalytically transfer electrons from DTT or AA to oxygen (O₂). DTT can~~
833 ~~be considered a chemical surrogate to cellular reductants, such as NADH or NADPH, which~~
834 ~~reduces O₂ to superoxide anion (O₂⁻) and induces oxidative stress (Kumagai et al., 2002). Unlike~~
835 ~~DTT, AA is a physiological antioxidant in lung lining fluid, which prevents the oxidation of~~
836 ~~lipids and proteins (Valko et al., 2005). Asthmatic patients have markedly decreased~~

837 ~~concentration of AA in lung lining fluid compared to healthy control subjects (Kelly et al.,~~
838 ~~1999). Therefore, the *in vitro* oxidation of these two antioxidants by PM might represent the~~
839 ~~interaction of PM with biological antioxidants within the body leading to the induction of~~
840 ~~oxidative stress and ultimately adverse health effects.~~

841 ~~It may be expected, however, that these two different assays respond to different aerosol~~
842 ~~components and are linked to different health endpoints. The AA assay has been shown to be~~
843 ~~most sensitive to transition metals (Janssen et al., 2014; Strak et al., 2012; DiStefano et al., 2009;~~
844 ~~Künzli et al., 2006) but quinone compounds may react with AA as well (Roginsky et al., 1999).~~
845 ~~For the DTT assay, identified DTT active PM components are organic species, including water-~~
846 ~~soluble organic carbon (WSOC) (Verma et al., 2009a; Cho et al., 2005), or of increasing~~
847 ~~specificity, Humic Like Substances (HULIS) (Verma et al., 2012; Lin and Yu, 2011), and~~
848 ~~quinones (Chung et al., 2006; Kumagai et al., 2002) (a component of HULIS). Other studies,~~
849 ~~however, have emphasized the role of transition metals, such as Cu and Mn (Vejerano et al.,~~
850 ~~2015; Charrier and Anastasio, 2012). Some differences between studies may arise due to~~
851 ~~differing source characteristics of the specific regions studied.~~

852 ~~Utilizing our automated analytical system (Fang et al., 2015b), we measured the water soluble~~
853 ~~oxidative potential of over 500 filter samples collected as part of the Southeastern Center for Air~~
854 ~~Pollution & Epidemiology (SCAPE) study using the DTT and AA assays. Here we evaluate and~~
855 ~~compare the two assays in order to identify the sources and specific aerosol components the~~
856 ~~assays respond to. We use the sources to generate a model that estimates DTT and AA activities~~
857 ~~and compare these estimated levels to the measured data. The model is then used to backcast~~
858 ~~DTT and AA levels over the past 10 years for use in a time-series epidemiological analysis in the~~
859 ~~Atlanta metropolitan area.~~

860 2. Methods

861 2.1. Sampling

862 Sampling methods have been described in detail ~~elsewhere in our previous publications~~ (Fang et
863 al., 2015b; Verma et al., 2014). In brief, PM_{2.5} (quartz filters, Pallflex® Tissuquartz™, 8 × 10
864 inches) was sampled at seven locations in the Southeastern US, with different source
865 characteristics, using two sets of high-volume samplers (Hi-Vol) (Thermo Anderson, flow rate
866 normally 1.13 m³ min⁻¹). Sampling in the metropolitan Atlanta area was carried out from June
867 2012 through March 2013 (noon - 11 a.m., 23 hours) and involved paired-sites with one Hi-Vol
868 sampler fixed at an urban background site (Jefferson Street, referred as JST) whilst the other
869 sampler was deployed at three other sites on a monthly basis, and at least twice during different
870 seasons. These three sites were: a rural site (Yorkville, YRK), a road-side site (RS, adjacent to
871 the interstate highway I75/85), and a near-road site (GT, 840 m from the RS site). Following
872 sampling in Atlanta, the two samplers were moved to Birmingham, AL (BHM, within a few
873 kilometers of significant transportation and industrial sources) and Centerville, AL (CTR,
874 surrounded by forests and a lightly traveled county road) for a month of sampling in June-July
875 2013, followed by ~~an~~ ~~one-month~~ August sampling at East St. Louis, IL, an urban residential/light
876 commercial area about 3 km east of the central business district of St. Louis, MO (Sauvain et al.,
877 2008). Finally, a GT-RS pair was conducted in September 2013. A table providing the sampling
878 schedule and a map can be found in the supporting materials (Table S1 & Fig. S1). JST, YRK,
879 BHM, and CTR are all part of the Southeastern Aerosol Research and Characterization Study
880 (SEARCH) network sites (Hansen et al., 2003). Collected samples were immediately wrapped in
881 prebaked aluminum foil and stored at -18°C until analyzed. DTT, water-soluble organic carbon,
882 and brown carbon analyses on the filters were conducted within a year of sample collection,

883 water-soluble elements were within a year and half, and AA measurements were conducted
884 within two years of sample collection.

885 2.2. Oxidative potential measurement

886 ~~2.2.1. Semi-automated system for measuring water-soluble DTT activity~~

887 ~~**Filter extraction:** Three punches of the collected Hi-Vol filter (5.07 cm² each) were extracted in
888 15 mL of deionized (DI) water ($> 18 \text{ M}\Omega \cdot \text{cm}^{-1}$) in a sterile polypropylene centrifuge tube (VWR
889 International LLC, Suwanee, GA, USA) by sonication using an Ultrasonic Cleanser (VWR
890 International LLC, West Chester, PA, USA) for half an hour. Extracts were then filtered using
891 PTFE 0.45 μm syringe filters (FisherbrandTM) to remove insoluble materials.~~

892 ~~**DTT determination:** The DTT assay was based on the protocol developed by Cho et al. (Cho et
893 al., 2005) and the semi-automated DTT system has been described in detail in Fang et al.
894 (2015b). In brief, 3.5 mL of the aerosol extract, 1 mL potassium phosphate buffer (Kbuffer, 0.5
895 mM), and 0.5 mL of DTT solution (1mM) were incubated at 37 °C in a continuously mixed vial
896 (400 rpm in a ThermoMixer (Eppendorf North America, Inc., Hauppauge, NY, USA)). At five
897 time intervals (4, 13, 23, 30, and 41 min), a small aliquot (100 μL) of the incubated mixture was
898 drawn to another vial and the DTT oxidation was quenched by adding 1 mL 1% w/v
899 Trichloroacetic acid (TCA). 0.5 mL of a color developing agent DTNB [5,5'-dithiobis-(2-
900 nitrobenzoic acid), 0.2 mM], with 2 mL of Tris buffer (0.08 M with 4 mM EDTA), was added to
901 form a light absorbing product, which has a high extinction coefficient at 412 nm wavelength.
902 Light absorbance at 412 nm and 700 nm (chosen as a baseline) for each time interval was
903 recorded and used to calculate the consumption rate of DTT over time ($\text{nmol} \cdot \text{min}^{-1}$). Final DTT
904 activity was reported as DTT consumption rate per unit of air volume ($\text{nmol} \cdot \text{min}^{-1} \cdot \text{m}^{-3}$), or per~~

905 unit of PM mass ($\text{nmol min}^{-1} \mu\text{g}^{-1}$). The automated system was cleaned periodically (typically
906 every 15 days) by flushing at least 3 times with methanol and then 6 times with DI water.
907 Sample selection, various chemical reagent addition and withdrawal, and system self-cleaning
908 were all achieved automatically by programmable syringe pumps (see details in section 2.2.2).

909 Final DTT activity is calculated as follows:

$$\sigma DTT = \sigma Abs \times \frac{N_0}{Abs_0} \quad (\text{Eq. 1}),$$

$$DTT = \frac{\sigma DTT_s - \sigma DTT_b}{\frac{V_s}{V_e} \times V_p} \quad (\text{Eq. 2}),$$

912 where σAbs is the slope of absorbance (412nm–700nm) versus time; Abs_0 is the initial
913 absorbance calculated from the intercept of linear regression of absorbance versus time; N_0 is the
914 initial moles of DTT added in the reaction (500 nmol); σDTT_s (σDTT_b) is the rate of DTT
915 consumption for sample (blank); V_e and V_s are the extraction volume (15 mL) and actual sample
916 volume added to the reaction vial (3.5 mL, i.e., what was actually analyzed), respectively. V_p is
917 the ambient air volume (m^3) represented by the sample in the extraction volume (V_e , i.e.,
918 considering the filter integration time and fraction of filter analyzed). DTT represents volume
919 normalized DTT activity, in units of $\text{nmol min}^{-1} \text{m}^{-3}$.

920 **2.2.2. Semi-automated system for measuring AA activity**

921 **Filter extraction:** One punch of the collected Hi-Vol filter (5.07 cm^2) was extracted in 30 mL of
922 deionized (DI) water ($> 18 \text{ M}\Omega \text{ cm}^{-1}$) in a sterile polypropylene centrifuge tube (VWR
923 International LLC, Suwanee, GA, USA) by sonication using an Ultrasonic Cleanser (VWR
924 International LLC, West Chester, PA, USA) for half an hour. For those having activities close to

925 blanks, 15 mL was used instead. Extracts were then filtered using PTFE 0.45 µm syringe filters
926 (Fisherbrand™) to remove insoluble material larger than 0.45 µm. Although OH may form
927 during sonication (Miljevic et al., 2014), it appears to have little effect on our ROS measurement
928 since we compared the water-soluble ROS activities from the same sample that had been
929 extracted by shaking for 3 hours vs sonication and found no significant differences (average ratio
930 and standard deviation is 1.08 ± 0.20 , $n = 7$).

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931 ~~Sample preparation protocol for the AA measurement is the same as that for the DTT assay,~~
932 ~~except that one punch of the filter (5.07 cm² each) was extracted in 30 mL of DI water, (for those~~
933 ~~having activities close to blanks, 15 mL of extraction DI was used instead).~~

934 **AA determination:** The method in this study was based on an ascorbate-only model (Mudway et
935 al., 2005; Ayres et al., 2008) ~~which that~~ is a simplified and alternative high throughput approach
936 to a synthetic respiratory tract lining fluid model (RTLFL) containing ascorbate, urate, and
937 reduced glutathione (GSH) (Zielinski et al., 1999; Mudway et al., 2004). The ~~semi-automated~~
938 ~~system for measuring AA activities was adapted from the automated DTT system discussed in~~
939 ~~section 2.2.1 method protocol is shown in Figure 1 and system setup can be found in the Figure.~~
940 ~~(Figs. S2 and S3S2 in the Supplement).~~ The method involves two steps.

941 The first step is an aerosol background measurement (Fig. ~~S2~~1). In order to control for the
942 contribution of absorbance of particles themselves at 265 nm wavelength, an AA-free control
943 was measured and subtracted from the sample absorbance readings. 2.4 mL aerosol extracts and
944 0.3 mL 0.5 mM Kbuffer were loaded into a reaction vial (~~Fig. S3~~)(sterile polypropylene
945 centrifuge tube, VWR International LLC, Suwanee, GA, USA) using a programmable syringe
946 pump (A) with a 5 mL syringe (Kloehn, Inc., Las Vegas, NV, USA) (~~Fig. S3~~). Following mixing,

947 90 μ L of the mixture was transferred to an intermediate vial using Pump B with a 250 μ L
948 syringe, and diluted to 3 mL. Pump A then withdrew the diluted mixture from the intermediate
949 vial and pushed it through a Liquid Wave-guide Capillary Cell (LWCC-M-100; World Precision
950 Instruments, Inc., FL, USA) with an optical path length of 100 mm. The waveguide was coupled
951 to an online spectrophotometer, which included a UV-VIS light source (Ocean Optics DT-Mini-
952 2, Ocean Optics, Inc., Dunedin, FL, USA), and a multi-wavelength light detector (USB4000
953 Miniature Fiber Optic Spectrometer, Ocean Optics, Inc., Dunedin, FL, USA). Aerosol
954 background absorbance at 265 and 700 nm (baseline) were recorded at two-second intervals
955 using data acquisition software (SpectraSuite). For the samples collected in this study,
956 backgrounds due to the aerosol absorption at 265 nm were <10% of the sample absorbance
957 readings. Prior to the second step, the system performed a self-cleaning by flushing the
958 intermediate vial and the two syringes with DI water three times.

959 The second step is the AA measurement (Fig. [S21](#)). Following the aerosol background
960 measurement, Pump A discarded a fraction of the sample-Kbuffer mixture and left only 1.8 mL
961 in the reaction vial. 0.2 mL 2 mM AA solution was then loaded to the reaction vial using Pump
962 B. Both the reaction and intermediate vial were continuously shaken at 400 rpm in a
963 ThermoMixer (Eppendorf North America, Inc., Hauppauge, NY, USA), which also maintained
964 the incubation temperature at 37 °C. At five different specified times (7, 15, 24, 32, 40 minutes),
965 a small aliquot (100 μ L) was transferred to the intermediate vial, diluted to 3 mL, and pushed
966 through the LWCC, generating a total of five data points quantifying the remaining AA
967 concentration. The system then again performed a self-cleaning before analyzing the next
968 sample. A multi-position valve (14-port, VICI® Valco Instrument Co. Inc., USA) was used to
969 select samples for analysis. To ensure the suspension of PM in the extract, each sample was

970 mixed by pushing 5mL of air through the extract before loading to the reaction vial. The detailed

971 Kloehn control program code for all steps can be found in the Supporting Information.

972 Final AA activity is calculated as follows:

973
$$\sigma AA = -\sigma Abs \times \frac{N_0}{Abs_0} \text{ (Eq. 31) ,}$$

974
$$AAv = \frac{\sigma AA_s - \sigma AA_b}{\frac{V_a}{V_e} \times V_p} \text{ (Eq. 42) .}$$

975 Following the notation above, σAbs is the slope of absorbance versus time, where the absorbance

976 is the absorbance of each time interval subtracting the corresponding aerosol background

977 absorbance; Abs_0 is the initial absorbance calculated from the intercept of linear regression of

978 absorbance versus time; N_0 is the initial moles of AA added in the reaction vial (400 nmol);

979 σAA_s (σAA_b) is the rate of AA consumption for a sample (blank); V_e and V_a are the extraction

980 volume (30 or 15 mL) and sample volume added to the reaction (1.6 mL), respectively. V_p is the

981 ambient air volume (m^3) represented by the sample in the extraction volume. AAv represents

982 volume normalized AA activity, in units of $nmol \text{ min}^{-1} m^{-3}$. Similarly, here DTTv represents the

983 volume normalized DTT activity.

984 2.3. Chemical analysis on PM filters

985 2.3.1. Water-soluble organic carbon and brown carbon

986 An automated system (details in Fig. S4S3) was used to measure water-soluble organic carbon

987 (WSOC) and brown carbon (BrC) on the water-soluble extracts from the same Hi-~~vol~~-Vol filters.

988 Filter extracts (~6mL, same extraction protocol outlined above), after loading onto a 5 mL

989 sample loop (Upchurch Scientific, Inc., Oak Harbor, WA), were first passed through a 1 m

990 ~~Liquid Wave guide Capillary Cell~~ LWCC (LWCC-2100; World Precision Instruments, Inc., FL,
991 USA), where absorbance at 365 nm wavelength (BrC) was measured using an online
992 spectrophotometer (Ocean Optics, Inc., Dunedin, FL, USA). The extracts then entered a TOC
993 analyzer (Sievers Model 900, GE Analytical Instruments, Boulder, CO, USA) for determining
994 WSOC concentration.

995 **2.3.2. Water-soluble elements**

996 A similar automated system was developed to determine the water-soluble elements, including S
997 (Sulfur), Ca (Calcium), K (Potassium), Fe (Iron), Cu (Copper), Zn (Zinc), Ba (Barium), Pb
998 (Lead), As (Arsenic), Sr (Strontium), Se (Selenium), Br (Bromine), Mn (Manganese), and Ti
999 (Titanium). Details of the method are described in Fang et al. (2015a) and in the Supplement.

1000 ~~Filter preparation protocol is the same as described above in section 2.2.1, except that four~~
1001 ~~punches were extracted in 15 mL of DI water, and after filtering, 120 μ L of high purity HNO_3~~
1002 ~~(OmniTrace® Ultra Nitric Acid, 67–70%, EMD Millipore Corporation, Billerica, MA, USA)~~
1003 ~~was then added to 6 mL of the extract (resulting pH \approx 0.7) to ensure the suspension of all~~
1004 ~~dissolved metals. Details of the method are described in Fang et al. (2015a). In brief, 5 mL of~~
1005 ~~acidified sample was aerosolized using a continuous flow ultrasonic nebulizer (CETAC U5000~~
1006 ~~AT+, CETAC Technologies, Omaha, NE, USA) and directed through an evaporator at 136 °C~~
1007 ~~followed by a condenser at 3 °C. The dry aerosolized sample was then neutralized by a Kr-85 ion~~
1008 ~~source (Model 3077A, TSI), mixed with filtered make-up air (final flow rate = 16.7 L min⁻¹, Pall~~
1009 ~~HEPA Capsule), and directed to an online aerosol element analyzer (Xact, Cooper~~
1010 ~~Environmental) for X-ray fluorescence (XRF) analysis.~~

1011 **2.3.3. PM_{2.5} mass**

1012 PM_{2.5} mass concentration was measured by a Tapered Element Oscillating Microbalance
1013 (TEOM) by Atmospheric Research Analysis (ARA, Inc.) at SEARCH sites (JST, YRK, BHM,
1014 and CTR) and ESL. For the RS and GT sites, ~~since PM_{2.5} mass were not available,~~ the PM mass
1015 concentrations were estimated from the sum of chemical components analyzed on the same Hi-
1016 Vol filters. ~~More details can be found in our other publications (Verma et al., 2014; Fang et al.,~~
1017 ~~2015b) (Details in the Supplement) and in associated supporting materials.~~

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1018 **2.4. Source apportionment**

1019 Source apportionment of ~~both DTT and AAv activities were~~was performed using a Positive
1020 Matrix Factorization (PMF) model (EPA PMF 5.0 software) (Paatero and Tapper, 1994) and a
1021 Chemical Mass Balance model (version 8.2) with ensemble-averaged source impact profiles
1022 (CMB-E) (Balachandran et al., 2012). PMF is a commonly used source apportionment approach
1023 that does not require source profiles as CMB-E, whereas CMB-E has better performance and
1024 lower relative uncertainties as compared to the PMF method (Balachandran et al., 2012). Source
1025 contributions to DTTv ~~activity~~ using PMF and CMB-E are discussed in our other publications
1026 (Bates et al., 2015; Verma et al., 2014). A PMF analysis on the water-soluble elements (S, K, Ca,
1027 Ti, Mn, Fe, Cu, Zn, As, Se, Br, Sr, Ba, and Pb) and WSOC from JST, GT, and RS sites has been
1028 reported in Fang et al. (2015a). ~~AAv was simply added to the data sets to generate the AAv~~
1029 ~~results shown here.~~
1030 ~~For the PMF analysis, water-soluble AA activity was added to the data sets. The PMF results on~~
1031 ~~DTTv in our prior analyses were based on JST and GT sites, used in prior analyses (Fang et al.,~~
1032 ~~2015a).~~

1033 In PMF, the uncertainties for each species were determined by multiplying the concentration by
1034 overall uncertainties (%), which were obtained by propagating the uncertainties from filter
1035 sampling (assumed to be 5%), extraction (assumed to be 5%), blanks (1 σ of multiple blanks),
1036 calibration (1 σ of slope, for water-soluble elements), collocated measurements (for water-
1037 soluble elements and AA, Fig. S5S4), and analytical uncertainties. The analytical uncertainties
1038 were obtained by analyzing the same sample/standards multiple times; for example, a composite
1039 of extracts from 11 samples for water-soluble elements (coefficient of variation, CV = 2-16 %);
1040 9,10-phenanthrenequinone for AA (CV = 13%), and sucrose standard solutions for WSOC (CV
1041 = 10%). Missing data were replaced by species medians with 400% uncertainty, and values
1042 below LOD were assigned as half of LOD values with uncertainties of 5/6 the concentration
1043 (Polissar et al., 1998). Uncertainty from collocated measurements was calculated as the relative
1044 uncertainty of the slope (1 σ /slope), which was based on an orthogonal regression.

1045 An ensemble-trained source apportionment approach (Balachandran et al., 2012) (CMB-E) was
1046 also used to construct the source impacts ~~of PM_{2.5} on DTT and AA_y activities~~ based on PM_{2.5}
1047 species (sulfate, nitrate, ammonium, OC, EC, and total metals) ~~and DTT/AA_y activity~~
1048 measured during SCAPE sampling periods (2012-2013). The source profiles cover a range of
1049 sources, including light-duty gasoline vehicles (LDGV), heavy-duty diesel vehicles (HDDV),
1050 ambient sulfate (AMSULF), ambient nitrate (AMNITR), ammonium bisulfate (AMBSLF), not
1051 otherwise apportioned organic carbon (OTHER_OC), dust, biomass burning (BURN), coal fired
1052 power plants ~~(CFPP)~~, cement ~~(CEM)~~, and cooking. An ensemble average was calculated for each
1053 source category using ten different runs developed from four individual source apportionment
1054 methods ~~(Balachandran et al., 2012)~~. Since the filters for the source impact profiles were
1055 collected from midnight to midnight, while the filters collected for ~~DTT and AA_y~~ measurement

1056 were collected from noon to 11 a.m. next day, the sources identified were linearly interpolated
1057 using a fixed ratio. For example, two consecutive filters (filter 1 and 2) ~~were~~ collected from
1058 midnight to midnight, ~~we would use~~ $12/24 * \text{filter1} + 11/24 * \text{filter2}$ would be used to produce the
1059 estimated ~~DTT or AA_y activities~~ to compared with actual measured data.

1060 2.5. Epidemiological assessment

1061 2.5.1. Backcast-estimates of AA ~~and DTT~~ activities

1062 To undertake a time-series epidemiological analysis with sufficient power, retrospective data sets
1063 of daily AA_y ~~and DTT~~ levels from 1 August 1998 to 31 December 2009 at an Atlanta site
1064 representative of the urban airshed air quality are needed. Previous epidemiological studies by
1065 the study team, assessing Atlanta air quality and emergency department (ED) visits, have used
1066 data from the SEARCH JST site, the anchor site for our ~~DTT and AA_y~~ measurements. To
1067 generate daily estimates of retrospective ~~DTT and AA_y activities~~ at JST, first a linear model was
1068 used to estimate the contribution of various sources to our observed AA_y ~~and DTT activities~~
1069 measured at JST (~~Bates et al., 2015~~). This was done through separate linear regressions for AA_y
1070 ~~and DTT activities on a per air volume basis~~, with the ensemble-predicted sources as
1071 independent variables. In previous work (Balachandran et al., 2012), a source times-series from
1072 August 1998 to December 2009 was generated for JST using the same CMB-E model with the
1073 same independent variables measured at JST. The ~~DTT and AA_y regressions were was~~ then
1074 applied to this time series to construct a time series of estimated ~~DTT and AA_y activities~~ for the
1075 epidemiology study time period, during which direct measurements of AA_y ~~or DTT~~ were not
1076 available. In order to test the sensitivity of epidemiologic results to different backcast models,

1077 two other models are generated for AAV (discussed in section 3.2.1). Identical methods were
1078 applied to DTTv to obtain three different models for comparisons with those from AAV.

1079 2.5.2. Epidemiological analyses

1080 Epidemiological time-series analysis (Strickland et al., 2010; Winquist et al., 2015) was
1081 employed to assess associations of retrospective DTTv and AAV ~~activities~~ with health effects as
1082 reflected in ED visits. Relationships between ~~The study team has published extensively on~~
1083 ~~previous work analyzing~~ ED visits data from Atlanta area hospitals ~~in relation to~~ and typical
1084 ambient air quality characteristics, as well as the impact of exposure misclassification and other
1085 factors, have been extensively studies (e.g., see [Strickland et al., 2015](#); [Darrow et al., 2014](#);
1086 [Strickland et al., 2014](#); [Wingquist et al., 2014](#); ~~-~~[Goldman et al., 2012](#); [Pachon et al., 2012](#);
1087 [Strickland et al., 2011](#); Strickland et al., 2010; [Sarnat et al., 2010](#); [Sarnat et al., 2008](#); [Tolbert et](#)
1088 [al., 2007](#); Metzger et al., 2004; Peel et al., 2005). For the present analysis, we apply ~~our~~ these
1089 previously reported epidemiologic modeling approach to the backcast-estimates of AAV and
1090 DTTv ~~activities~~, in order to assess associations of these newly developed air quality descriptors
1091 with selected outcomes in the ED visits data collected from hospitals serving the five-county
1092 metropolitan Atlanta area during 1998-2009. The health outcomes investigated in the current
1093 analysis are daily visits for respiratory diseases, including pneumonia (n=145,610 total visits for
1094 study period), chronic obstructive pulmonary disease (n=49,251), and asthma/wheeze
1095 (n=263,665), and cardiovascular diseases, including ischemic heart disease (n=73,477) and
1096 congestive heart failure (CHF) (n=70,587). The air quality was modeled as a three-day moving
1097 average (“lag 0-2”, the moving average of estimated pollutant level for that day, the previous
1098 day, and the day before). Poisson generalized linear regression was performed; to control for
1099 temporal trends and meteorological variables, models included cubic splines with monthly knots

1100 for time, linear, quadratic and cubic terms for mean daily dew point (lag 0-2), maximum daily
1101 temperature (lag 0), and minimum daily temperature (lag 1-2), indicators of hospital contribution
1102 time periods, season of year, day of week and holiday, and interaction terms between season and
1103 maximum temperature, and between season and day of week. These covariates were chosen
1104 based on prior studies (Strickland et al., 2010; Winqvist et al., 2015) which identified important
1105 confounders to the relationship between daily ambient pollution levels and ED visits. Risk ratios
1106 (the relative risks of ED visit associated with an increase of one interquartile range of the
1107 exposure metric) and 95% confidence intervals (CI) were used to describe the observed health
1108 associations. Risk ratios with confidence intervals above 1 are indicative of statistically
1109 significant positive associations. International classification of disease codes used to define the
1110 health outcomes can be found in the ~~supplement~~Supplement.

1111 **3. Results and ~~discussion~~Discussion**

1112 **3.1. ~~Comparing DTT and AA based on measurement during 2012-2013~~AAv for** 1113 **~~measurements during 2012-2013 and comparisons to DTTv~~**

1114 **3.1.1. Spatio-temporal distribution**

1115 Monthly average water-soluble ~~AAv and DTT activities~~ at various sampling sites are given in
1116 Fig. ~~1-2~~ (a) ~~and (b), respectively. There were differences in the seasonal and spatial patterns~~
1117 ~~between AA and DTT activities in the Southeastern US. Fig. 1 shows that AAv was~~
1118 ~~heterogeneously distributed, indicated by the significant variability between sites. Highest AAv~~
1119 ~~were found at the roadside site (RS) and lowest at rural sites. For example, the ratio of average~~
1120 ~~AAv at RS to its paired Atlanta urban JST site, was 1.2 in fall and 1.6 in winter [(2.7 when~~
1121 ~~including the four high data points in Fig. 2 (a)] and RS to near-road GT was 1.7 in fall 2013.~~
1122 ~~AAv at the rural site was generally lower compared to the urban environments, the average~~

1123 YRK/JST ratio was 0.7 in summer and 0.4 in winter, respectively. An exception is that BHM
1124 (urban) and CTR (rural) had similar AAV ($BHM_{avg} = 0.75 \pm 0.45 \text{ nmol min}^{-1} \text{ m}^{-3}$ and $CTR_{avg} =$
1125 $0.78 \pm 0.31 \text{ nmol min}^{-1} \text{ m}^{-3}$). Comparing AAV at different sites, BHM and the other urban site,
1126 ESL (average AAV= $0.98 \pm 0.63 \text{ nmol min}^{-1} \text{ m}^{-3}$), had lower AAV relative to the Atlanta urban
1127 sites (average of JST and GT in summer = $2.5 \pm 1.0 \text{ nmol min}^{-1} \text{ m}^{-3}$). The higher AAV near
1128 traffic sources has also been found in other studies (Janssen et al., 2014; Strak et al., 2012;
1129 Janssen et al., 2015). In contrast, Figure 2(b) shows that DTV activity was largely spatially
1130 uniform, differences between paired sites is much less than those for AAV, except the JST-YRK
1131 pair in winter (December) ($DTV_{at JST} > DTV_{at YRK}$) (also discussed in previous publications
1132 (Verma et al., 2014; Fang et al., 2015b)), while AA was heterogeneously distributed, with the
1133 highest levels near highly trafficked highways and lowest at rural sites. For example, the ratio of
1134 averaged AA activity at RS to its paired Atlanta urban JST site, was 1.2 in fall and 1.6 in winter
1135 [(2.7 when including the four high data points in Fig. 1(a)] and RS to near road GT was 1.7 in
1136 fall 2013. AA was generally lower in rural compared to urban environments (ratio of YRK/JST
1137 is 0.7 in summer and 0.4 in winter, respectively). An exception is that BHM (urban) and CTR
1138 (rural) had similar AA levels ($BHM_{avg} = 0.75 \pm 0.45 \text{ nmol min}^{-1} \text{ m}^{-3}$ and $CTR_{avg} = 0.78 \pm 0.31$
1139 $\text{nmol min}^{-1} \text{ m}^{-3}$). BHM and the other urban site, ESL (average AA= $0.98 \pm 0.63 \text{ nmol min}^{-1} \text{ m}^{-3}$),
1140 had lower AA activities relative to the Atlanta urban sites (average of JST and GT in summer =
1141 $2.5 \pm 1.0 \text{ nmol min}^{-1} \text{ m}^{-3}$). The higher AA activities near traffic sources have also been found in
1142 other studies (Janssen et al., 2014; Strak et al., 2012; Janssen et al., 2015).

1143 The seasonal distribution can be examined from the Atlanta sites, JST, GT, RS, and YRK. The
1144 AA activity was higher in summer/fall compared to winter; the ratio of summer or fall to winter
1145 was 1.8, 2.9, 1.0, and 3.1 (average ratio is 2.2 ± 0.9) for JST, YRK, RS, and GT, respectively. In

1146 ~~contrast, JST. As discussed in our previous work (Verma et al., 2014; Fang et al., 2015b), there~~
1147 ~~was high heterogeneity in DTT levels across seasons. DTTv had an opposite seasonal trend, at~~
1148 ~~JST with the highest level in December (winter), while there was no significant seasonal~~
1149 ~~variation observed at YRK, GT and the RS site (also seen in Fig. 1). In contrast, the AA activity~~
1150 ~~was higher in summer/fall compared to winter; the ratio of summer or fall to winter was 1.8, 2.9,~~
1151 ~~1.0, and 3.1 (average ratio is 2.2 ± 0.9) for JST, YRK, RS, and GT, respectively. These results~~
1152 ~~indicate that there are differences in the sources for water-soluble AAv and DTTv, with traffic~~
1153 ~~emissions a more significant source for AAv. Correlation analysis with specific aerosol~~
1154 ~~components provides further insights. These results indicate that water-soluble DTT and AA~~
1155 ~~activities of PM_{2.5} were influenced by different aerosol components from differing sources that~~
1156 ~~varied with season.~~

1157 **3.1.2. Correlations with chemical components**

1158 ~~As an initial step to identify~~To further identify the major sources for ~~DTT and AAv and compare~~
1159 ~~to DTTv activities~~, a correlation analysis was performed between the assays and ~~the following~~
1160 selected chemical components, ~~including~~: BrC (~~an indicator of incomplete combustion, i.e., a~~
1161 biomass burning ~~indicator~~), WSOC and S (secondary processes), Ca (mineral dust), and selected
1162 transition metals (Cu, Fe, Mn, and Zn) that have been related to adverse health outcomes
1163 (Cheung et al., 2012; Kam et al., 2011; Shen and Anastasio, 2011; Cheung et al., 2010; Akhtar et
1164 al., 2010; Landreman et al., 2008; Zhang et al., 2008; Kodavanti et al., 2005). Correlation
1165 coefficients based on linear regressions between ~~AAv, DTT or DTTv, AA activity~~ and chemical
1166 species (Pearson's r) are shown graphically in Fig. ~~23~~. A detailed matrix showing the
1167 correlations at individual sites is given in Table S2. To simplify Figure ~~23~~, JST and GT were
1168 combined into one metric given their close proximity and high correlation ($r^2 > 0.57$) ~~reported in~~

1169 ~~our previous work~~ for many PM species, such as ~~ECC~~ (Verma et al., 2014), ~~WSOC~~ (Verma et
1170 ~~al., 2014~~), and water-soluble elements (Fang et al., 2015a; Verma et al., 2014). As shown in Fig.
1171 ~~23~~, ~~AAv~~ was almost exclusively correlated with water-soluble Cu. The r value ranged from 0.70-
1172 0.94 for most sites/seasons except RS in fall 2012, JST/GT in winter, ESL in summer, and GT in
1173 fall 2013. High correlations between AAv and Cu are consistent with other studies (Janssen et
1174 al., 2014; Künzli et al., 2006), although the correlation coefficients (r) in our work (0.70-0.94)
1175 were higher (0.60-0.74 in other studies), possibly because we used water-soluble Cu and the
1176 other studies used elemental (total) concentrations. Strak et al (2012) also reports a higher r value
1177 between AAv and water-soluble Cu (r = 0.82) than that between AAv and total Cu (r = 0.76)
1178 from the same sample set.

1179 ~~Compared to AAv, DTTv is more broadly correlated with aerosol species: high correlations were~~
1180 ~~observed DTT activity was correlated with S (r = 0.7 at JST/GT, 0.74 at YRK, 0.66 at BHM66-~~
1181 ~~0.74) and WSOC (r = 0.71 at JST/GT, 0.74 at YRK, 71- 0.77 at CTR) in summer, which~~
1182 ~~diminished in fall (r = 0.14-0.66 for S and r = 0.20-0.65 for WSOC) and was weaker in winter~~
1183 ~~(r<0.4) for S. Instead, higher correlations were found with BrC (r = 0.78-0.88) and WSOC (r =~~
1184 ~~0.60-0.84) in winter. The decreasing correlation between DTTv and S going from summer to~~
1185 ~~winter suggests the important role of secondary processing in summer (Verma et al., 2009a;~~
1186 ~~McWhinney et al., 2013) and suggesting the important role of secondary processing (Verma et~~
1187 ~~al., 2009a; McWhinney et al., 2013). These correlations diminished in fall (r = 0.14-0.66 for S~~
1188 ~~and r = 0.20-0.65 for WSOC) and weak correlations were observed in winter (r<0.4) for S.~~
1189 ~~Instead, higher correlations were found between DTT and BrC (r = 0.78-0.88) and WSOC (r =~~
1190 ~~0.60-0.84) in winter. This suggests an~~ the increasing contribution of biomass burning to winter
1191 DTTv activities (Verma et al., 2009b); ~~AAv~~ did not show similar trends, i.e., AAv did not

1192 ~~correlate with S ($r = -0.12-0.60$) and low r values were observed with K in winter ($r = 0.07-0.19$,~~
1193 ~~one exception was JST in December $r = 0.7$), suggesting incomplete combustion (e.g., biomass~~
1194 ~~burning) was not a significant source for AAv. Whereas AAv was nearly exclusively correlated~~
1195 ~~with Cu, DTTv was correlated with various metals, including Ca, Mn, Fe, Cu, and Zn. In Fig. 2,~~
1196 ~~counting the number of sampling sites at which r values between AAv and various metals were~~
1197 ~~larger than 0.65 (i.e. black solid bars), we observed eight times for Cu and once for Mn ($r =$~~
1198 ~~0.82). Whereas for DTTv (see the striped bars in Fig.3), we found twice ($r = 0.67$ and 0.77) for~~
1199 ~~Ca, 3 times for Mn ($r = 0.65-0.75$), 6 times for Fe ($r = 0.68-0.90$), once for Cu ($r = 0.68$), and 3~~
1200 ~~times for Zn ($r = 0.70-0.82$). DTT activities were also correlated with various metals, including~~
1201 ~~Ca ($r = 0.67-0.77$), Mn ($r = 0.65-0.75$), Fe ($r = 0.65-0.74$), Cu ($r = 0.68$), and Zn ($r = 0.76-0.82$),~~
1202 ~~but there were, however, no apparent seasonal patterns for the correlations between these~~
1203 ~~water-soluble metals DTT and DTTv these water-soluble metals since they were related to~~
1204 mineral dust (68% of Ca, 45% of Mn, and 26% of Fe) and vehicle brake/tire wear emissions
1205 (51% of Cu, 45% of Zn, 32% of Fe, and 17% of Mn), as reported by Fang et al. (2015a).

1206 ~~The comparison of AAv and DTTv's correlation with $PM_{2.5}$ mass is noteworthy. In contrast, AA~~
1207 ~~has a much narrower sensitivity to aerosol species than DTT in that it was only correlated with~~
1208 ~~water-soluble Cu ($r = 0.70-0.94$ for most sites/seasons except JST in Feb, GT in March, and~~
1209 ~~GT/RS in Sept 2013) (Fig. 2) in the Southeastern US. High correlations between AA and Cu are~~
1210 ~~consistent with other studies (Janssen et al., 2014; Künzli et al., 2006), although the correlation~~
1211 ~~coefficients (r) in our work (0.70-0.94) were higher (0.60-0.74 in other studies), possibly because~~
1212 ~~we used water-soluble Cu and the other studies used elemental (total) concentrations. Strak et al~~
1213 ~~(2012) also reports a higher r value between AA and water-soluble Cu ($r = 0.82$) than that~~
1214 ~~between AA and total Cu ($r = 0.76$) from the same sample set.~~

1215 DTT_v activities (per volume) were fairly well correlated with PM_{2.5} mass (r = 0.49-0.86, Fig.
1216 32), although the slope of DTT over PM_{2.5} mass differed by season likely due to different
1217 contributing source mixtures (Fang et al., 2015b)), whereas In contrast, AA_v did not correlate
1218 as well with PM_{2.5} mass (r < 0.58 = -0.17 to 0.59), as also found in Künzli et al. (2006) (r = 0.34).
1219 PM_{2.5} mass has been linked with adverse health endpoints in many epidemiological studies
1220 (Laden et al., 2000; Pope et al., 2002; Pope et al., 2004; Metzger et al., 2004; Sarnat et al., 2008),
1221 thus the lack of correlation of DTT-AA_v with PM_{2.5} mass suggests may be suggestive of a lack
1222 of linkage between DTT-AA_v-measured oxidative potential and health endpoints (tested in section
1223 3.2). In summary, AA and DTT assays have differing responses-associations to-with PM
1224 chemical species, further suggested by a lack of strong correlation between the two assays at all
1225 sites (r < 0.55, Fig. 2), similar to findings from other studies (r < 0.65) (Janssen et al., 2014; Yang
1226 et al., 2014; Janssen et al., 2015). A source apportionment analysis is performed to attempt to
1227 quantify contributions of various sources to PM_{2.5} AA_v.

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1228 3.1.3. Source apportionment

1229 Various source contributions to water-soluble AA_v and DTT_v activities resolved from PMF and
1230 CMB-E based on measured data during 2012-2013 are shown in Fig. 3-4 (a, b) and (c, d),
1231 respectively. For water-soluble AA activity AA_v, the detailed loadings of various species and
1232 time series of each factor suggested by PMF can be found in Fig. S6S5.

1233 **AA Sources:** Comparing Fig. 3-4 (a) and (b) shows that CMB-E and PMF gave consistent and
1234 complementary results for AA_v sources. Consistent with the spatial distributions, CMB-E
1235 indicated that vehicles comprise almost half of the total source contributions to PM_{2.5} AA_v
1236 activity with roughly equal contributions from light and heavy duty vehicles. PMF also found
1237 vehicle emissions as a major source, but resolved the source as mechanical generation processes

1238 (44% from brake/tire wear). Both methods also found sources of AA_v associated with secondary
1239 processes. CMB-E provides more details in that it separated out secondary organic and ambient
1240 sulfate sources. This is consistent with our source apportionment analyses on water-soluble
1241 metals (Fang et al., 2015a), which showed that Cu was mainly associated with secondary
1242 formation and brake/tire wear, consistent with AA_v being highly correlated with Cu. The 19%
1243 other OC source in Figure 3b is related to un-apportioned OC, which -and so- includes mainly
1244 secondary biogenic-organic aerosols from biogenic emissions, and possible additional
1245 contributions from other VOC sources. At this point, the role it plays as a source of AA_v is not
1246 clear. It should be noted that the residual for PMF was -10% and that for CMB-E was 14%,
1247 which means the PMF model over-predicted and CMB-E under-estimated AA_v activity. The
1248 source apportionment analysis is consistent with the spatial distribution, which indicated vehicle
1249 emissions as a main source for AA_v activity.

1250 **AA_v compared to DTT_v Sources:** Comparisons of the ~~The~~ source apportionment results on
1251 water-soluble ~~AA_v activity~~ using PMF ~~(Verma et al., 2014)~~ and CMB-E to a similar
1252 analyses for DTT_v is insightful ~~were compared to those for AA~~. PMF source apportionment
1253 analyses [Fig. ~~34~~(a) and (c)] suggest a common contribution from traffic emissions and
1254 secondary processes to both water-soluble ~~AA_v DTT~~ and ~~DTT_v AA~~ activities, but the
1255 contributions were stronger for AA_v than DTT_v. For example, 44% AA_v activity was attributed
1256 to vehicles and 56% to secondary processes, compared to 16% and 31% ~~of for DTT_v activities~~,
1257 respectively. Higher fractional contributions of these two sources for AA_v is because unlike
1258 DTT_v, biomass burning does not contribute to AA_v (1%), whereas it makes a large contribution
1259 to the overall study DTT_v (35%). CMB-E also found no contribution of biomass burning to
1260 AA_v, but identified a fractional contribution from biomass burning [36% BURN in Fig. 4(d)] to

1261 DTTv similar to PMF. This is because a large fraction of the DTT activities (35%) were related to
1262 biomass burning while only 1% of AA activities were attributed to biomass burning. CMB-E
1263 also found similar contribution from biomass burning [36% BURN in Fig. 3(d)] to DTT
1264 activities. CMB-E points to ammonium sulfate (AMSULF) as a source for both AAv and DTTv.
1265 Neither AA nor DTT assay responds to pure ammonium sulfate, meaning that ammonium sulfate
1266 is an indicator of some source or process. It may be a marker for atmospheric processed or aged
1267 aerosols. For example, both assays respond to water-soluble transition metals, and a significant
1268 fraction these metals, when emitted, are not water-soluble [solubilities of Zn is ~50%, Cu and
1269 Mn 10-40%, Fe < 10% (Birmili et al., 2006; Espinosa et al., 2002)]. Mobilization by acidic
1270 aerosols can increase the soluble fraction, which requires a low aerosol pH and time, both can be
1271 linked to sulfate aerosol. For example, at pH < 2, 1-2% of mineral dust Fe is mobilized within 3-
1272 5 days (Meskhidze et al., 2003).

1273 It is worth noting that both assays appear to be linked to emissions from traffic, but the actual
1274 sources from traffic differ. AAv was almost exclusively associated with mechanically generated
1275 aerosols (i.e. brake/tire wear), whereas for water-soluble DTTv, traffic emissions included both
1276 metals and organic aerosol species, i.e., from mechanically generated (brake/tire wear) and
1277 combustion (tail pipe emissions). Finally, correlations to specific aerosol species and source
1278 apportionment analysis can be confounded by co-variability with other unmeasured components
1279 or processes, as demonstrated by the associations with ammonium sulfate, or nonlinear responses
1280 of these assays to specific components (Charrier et al., 2015). However, the major sources
1281 identified for both AAv and DTTv, and the contrasts between their sources, is consistent with the
1282 season trends and spatial distributions observed and discussed above for each assay, indicating
1283 that the source apportionment analysis is robust. The common contribution of ammonium sulfate

1284 (*AMSULF*) to *DTT* and *AA* may represent different sources, i.e., organic aerosol oxidation or
1285 metal mobilization by formation of an aqueous particle with secondary acids. Note that for
1286 water-soluble *DTT*, traffic emissions included both metals and organic aerosol species, that is
1287 from mechanically generated (brake/tire wear) and combustion (tail pipe emissions),
1288 respectively, whereas *AA* was almost exclusively associated with mechanically generated
1289 aerosols (i.e. brake/tire wear).

1290 **3.2. *AA_v* and *DTT* activities compared to association with health endpoints and application** 1291 **to epidemiological models contrasts to *DTT_v***

1292 **3.2.1. Backcast-estimates of *AA_v* and *DTT* activities using Source Impacts**

1293 Although over roughly 1 year of *AA_v* of *DTT* and *AA* activities were generated for the central
1294 JST site in Atlanta, longer data sets are generally needed for a time series epidemiological study.

1295 To generate these data, a multiple linear regressions were used to estimate *AA_v* and *DTT*
1296 activities from the CMB-E identified sources. We follow the same approach as that used for
1297 *DTT_v* (Bates et al., 2015). Water-soluble *AA_v* and *DTT* activities (nmol min⁻¹ m⁻³) measured
1298 between 2012 and 2013 at JST were regressed against all CMB-E sources. Insignificant sources
1299 (p of F-statistic of coefficient > 0.05, Table S3) and the significant sources with negative
1300 coefficients were removed. The latter occurred for BURN (biomass burning) and AMNTR
1301 (ammonium nitrate) in the *AA* regression, likely due to their opposite seasonal trends to the
1302 measured *AA_v* activity. These two sources also did not contribute to *AA_v* [see Fig. 4(a)] Biomass
1303 burning contributed only 1% and ammonium nitrate did not contribute to *AA* activity [see Fig.
1304 3(a)]. The final regressions for *AA_v* and *DTT* activities are:

$$1305 \text{AAAA}_v = 0.079 + 0.19 \text{LDGV} + 0.23 \text{HDDV} + 0.063 \text{AMSULF} + 0.075 \text{OTHER_OC} \quad (\text{Eq. 53})$$

1306 For direct comparison with DTT_v, we used the same criteria for including various sources in the
1307 DTT_v regression model, with the result:

1308

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1309 ~~DTT~~DTT_v^e = 0.067 + 0.11 LDGV + 0.045 HDDV + 0.02 AMSULF + 0.069 BURN (Eq. 64)

1310 (Note, the DTT_v^e regression is different from that in Bates et al., (2015) in that AMSULF (ammonium
1311 sulfate) was included in this model)

1312 ~~AA_v^e, where DTT~~ and DTT_v^eAA are the estimated ROS activities of PM_{2.5} (nmol min⁻¹ m⁻³), which
1313 are related to the following sources (μg m⁻³): light-duty gasoline vehicles (LDGV), heavy-duty
1314 diesel vehicles (HDDV), ammonium sulfate (AMSULF), biomass burning (BURN), and other
1315 organic carbon (OTHER_OC). The coefficients in the equations represent the intrinsic activities
1316 (nmol min⁻¹ μg⁻¹) of the sources, a measure of the strength of the source on a per PM_{2.5} mass
1317 basis for water-soluble AA_v^e or DTT_v^e activities. Interestingly, for both assays, the traffic sources
1318 (LDGV+HDDV) has the highest ROS intrinsic activity, while secondary sources or biomass
1319 burning have relatively lower ROS intrinsic activities. The high intrinsic activity in the traffic
1320 sources might be attributed to metals that have much higher intrinsic ROS activities~~The high~~
1321 ~~DTT intrinsic activity in the traffic source might be attributed to metals that have much higher~~
1322 ~~intrinsic DTT activities~~ (Charrier and Anastasio, 2012; Verma et al., 2015). The much higher
1323 coefficients of LDGV and HDDV in the AA_v^e regression than those in DTT_v^e highlight the larger
1324 role of metals from these sources contributing to the overall AA_v^e. ~~AA~~ Although biomass burning
1325 has a lower DTT intrinsic activity compared to the other sources in the DTT_v^e model, it was the
1326 largest contributor to DTT_v^e ~~DTT activity~~ due to the strength of this source over the measurement
1327 period (e.g. large magnitude of BURN). ~~(Bates et al., 2015).~~ ~~For AA, vehicles and secondary~~

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1328 formation had the largest contribution. The regression positive intercepts (0.079 and 0.067 for
1329 AA and DTF regression, respectively) indicate some levels of discrepancy between the
1330 model and measurement unidentified source for AA_v and DTT_v. The ability of the models to
1331 predict AA_v^e and DTT_v^e is given by the correlation between the model and measurements (AA_v^e vs
1332 AA_v and DTT_v^e vs DTT_v). The r² values (are 0.60 and 0.68 for AA_v^e and DTT_v^e,
1333 respectively) between measured and predicted activities represent the uncertainties, indicating
1334 the models can only account for about 40% of the observed variability. Regression coefficients,
1335 p-values, and the correlation coefficients (r) values are summarized between measured and
1336 estimated data in Table S3.

1337 To test the sensitivity of the epidemiological results to other predictive models, two other
1338 regressions were used to predict AA_v and DTT_v: 1) all sources included, 2) only
1339 significant sources with positive coefficients (above), but with AMSULF removed (summarized
1340 in Table S3). The latter was done because sulfate has substantially decreased over the last decade
1341 due to emission reductions in the southeastern US (Hidy et al., 2014; Hand et al., 2012), which
1342 may have unknown effects on AA_v^e and DTT_v^e. These models and various statistics are also
1343 summarized in Table S3. For both assays the models with all sources included had highest
1344 correlations coefficients between model and observed activities (r ~ 0.7, or model explains ~50%
1345 of the variability).

1346 3.2.2. Health associations from time-series epidemiological models

1347 Backcast AA_v^e and DTT_v^e activities were next generated for the study period
1348 corresponding to the health analysis (ED) data. The various regression models (including from
1349 Eq. 5-3 and 6-4) were used to generate daily retrospective estimates of AA_v and DTT_v activities
1350 at the JST site for the period of 1998-2009 (Bates et al., 2015), based on existing source impacts

1351 generated in a previous study ~~for the same site in the Southeastern US (Balachandran et al.,~~
1352 ~~2012). Estimated water-soluble-AA_v^e-DTT and DTT_v^e-AA activities~~ were run separately in
1353 epidemiological models of ED visits for selected outcomes (section 2.5.2). The risk ratios for
1354 ~~AA_v^e-DTT and DTT_v^e-AA activities~~ for asthma/wheeze and congestive heart failure are presented
1355 in Figure ~~4-5~~ (data given in Table S4). The other health outcomes (chronic obstructive
1356 pulmonary disease, pneumonia, and ischemic heart disease) did not show significant associations
1357 with ~~AA_v^e estimated-DTT or DTT_v^e AA activity~~ (results ~~given in Table S4~~ not presented).

1358 For asthma/wheeze and congestive heart failure, although the risk ratios for an increase of an
1359 interquartile range for ~~DTT-AA_v^e estimated from the model (Eq.6)~~ were above 1 [~~(Fig. 4-1.005 and~~
1360 ~~1.003 for Asthma/wheeze and CHF, respectively, Fig. 5(a))~~], ~~and~~ the 95 % confidence intervals
1361 ~~exclude~~ crossed 1 (0.994-1.015 and 0.986-1.020 for Asthma/wheeze and congestive heart failure,
1362 respectively), indicating a non-statistically significant ~~positive~~ association between AA_v^e
1363 ~~estimated-DTT~~ and the ED visits for these health outcomes. In contrast, ~~neither both~~ of the health
1364 outcomes showed statistically significant associations with the ~~DTT_v^e AA estimates. Similar-The~~
1365 ~~same results~~ differences were found for estimates based on the two other regressions [~~(see Figure~~
1366 ~~S75(b) and (c))~~], suggesting that the null relationship of AA_v^e and positive association of DTT_v^e
1367 with these health outcomes are to some extent robust, despite the high uncertainties from the
1368 back-cast models. -A possible cause for the differences in ~~DTT-AA_v^e and AA-DTT_v^e health~~
1369 associations is, at least ~~differences in uncertainties in the estimated historical ROS activities; the~~
1370 ~~model did not observed AA activities as well as DTT, (observed vs predicted r values 0.60 and~~
1371 ~~0.68 for AA and DTT, respectively). F~~or this study region, the more narrow selectivity of the
1372 AA assay to specific aerosol components (i.e., mostly sensitive to Cu). ~~The may result in AA~~

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1373 ~~assay may not capturing-capture the integrated-overall oxidative potential of all the various PM~~
1374 ~~ROS activity of all the various PM~~ components as well as the DTT assay-
1375 PM-induced oxidative stress in the exacerbation of asthma (Li et al., 2003) and the
1376 cardiovascular system (Donaldson et al., 2001) has been proposed. Linkages seen here between
1377 water-soluble ROS activity and morbidity due to asthma/wheeze and congestive heart failure are
1378 consistent with these studies and the contrast with the AAv results suggest the importance of
1379 organic components and transition metals from biomass burning and vehicular emissions in the
1380 Southeastern US, and support aerosol particle oxidative potential as a mechanism contributing to
1381 these PM-induced adverse health effects. Although some studies have shown that ROS plays a
1382 key role in COPD (O'Donnell et al., 2006), IHF (Lakshmi et al., 2009; Giordano, 2005), and
1383 Pneumonia (Kuwano et al., 2003), we did not observed a significantly positive association of the
1384 tested ROS assays with these health outcomes. Finally, although this work shows a contrast
1385 between these two assays and association with health endpoints, Janssen et al. (2015) found
1386 significant associations between both assays and nasal and airway inflammation based on a
1387 different approach (panel study, n=31).

1388 4. Summary

1389 Approximately 500 PM_{2.5} high-volume filter samples collected in the Southeastern US were
1390 analyzed for aerosol oxidative potential using the ~~DTT and~~ AA assays. The AA activities
1391 reported are from the same filters for which water-soluble DTT activities had already been
1392 determined. We found that water-soluble AA activity on a per air volume basis (AAv) was
1393 highest near roadways and lowest at rural sites. AAv was higher in summer/fall than winter.
1394 These results are in contrast to DTTv, which was more spatially uniform and had an opposite

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1395 ~~seasonal trend at the urban Atlanta site (higher in winter than summer/fall). water-soluble DTF~~
1396 ~~activity ($\text{nmol min}^{-1} \text{m}^{-3}$) was fairly spatially uniform and higher in winter than in summer/fall at~~
1397 ~~the urban Atlanta site, whereas AAv was most consistently correlated with water-soluble metals~~
1398 ~~(especially water-soluble Cu), whereas water-soluble AA activity had highest levels near~~
1399 ~~roadways, and an opposite seasonal trend (higher in summer/fall than winter). DTTv activity was~~
1400 ~~correlated with organic species, and water-soluble metals (Fe, Cu, Zn, Mn, and Ca), and also~~
1401 ~~PM_{2.5} mass, whereas AA activity was only correlated with water-soluble metals (especially~~
1402 ~~water-soluble Cu). A Source apportionment analysis suggest indicated that traffic emissions~~
1403 ~~secondary processes and secondary processes traffic emissions were strong contributors to both~~
1404 ~~AAv DTF and DTTv AA activities in urban Atlanta, GA, with traffic emissions the most~~
1405 ~~important. For AAv only road dust was responsible, in contrast to both combustion emissions~~
1406 ~~and road dust contributing to the DTTv from this source. Biomass burning was did not contribute~~
1407 ~~to AAv, but was a large-substantial source for DTTv activity, but insignificant for AA consistent~~
1408 ~~with AAv being mainly associated with transition metals. These source apportionment results are~~
1409 ~~also consistent with observed seasonal trends and spatial distributions, for both assays. Multiple~~
1410 ~~linear regressions suggest that mobile sources have the highest ROS intrinsic activities (i.e.,~~
1411 ~~activities per PM_{2.5} mass from that source) for both assays. Time-series large population~~
1412 ~~epidemiological analyses using baekast-backcast-estimates of AAv DTF and AA-DTTv from a~~
1413 ~~number of linear models activities-based on 10-year historical source impacts suggest that DTF~~
1414 ~~AAv activity was not associated-linked with any emergency department (ED) visits for both~~
1415 ~~asthma/wheeze and congestive heart failure all tested health outcomes at 95% confidence~~
1416 ~~intervals. DTTv was associated with ED visits for both asthma/wheeze and congestive heart~~
1417 ~~failure, for all the linear models tested. Neither AAv nor DTTv was associated with chronic~~

1418 obstructive pulmonary disease (COPD), Ischemic heart failure (IHD) or pneumonia at a
1419 statistically significant level. Based on the wide-ranging comparisons between these assays, we
1420 conclude that, while AA activity was not linked to any health outcomes. For the region
1421 investigated in this study, the DTT assay was a more comprehensive multi-pollutant indicator of
1422 ROS (or PM_{2.5} oxidative potential) indicator than the AA assay. This can be useful when deciding
1423 on what assay to utilize to address the goal of a specific study. making DTT is a potentially a
1424 more valuable parameter to include in future PM health-related studies because of its broader
1425 sensitivity to aerosol components associated with oxidative potential. Finally, The ability to
1426 readily measure both PM_{2.5} AA and DTT with automated systems enables activities can support
1427 further larger scale studies of involving direct measurements of PM oxidative potential. These
1428 types of future studies are needed to test these measures and linkages to health outcomes to assess
1429 if these our health findings based on backcast-estimated AA and DTT levels are robust and
1430 applicable to found in other regions.

1431

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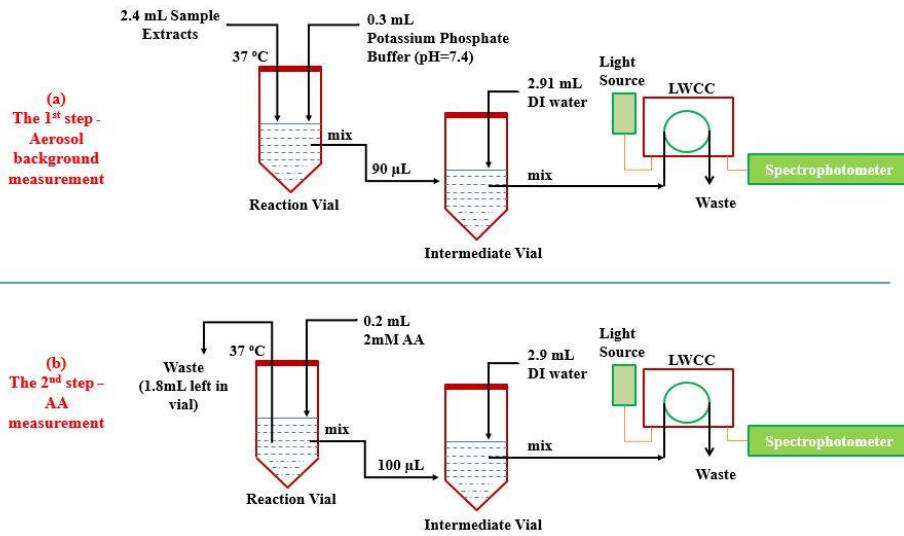
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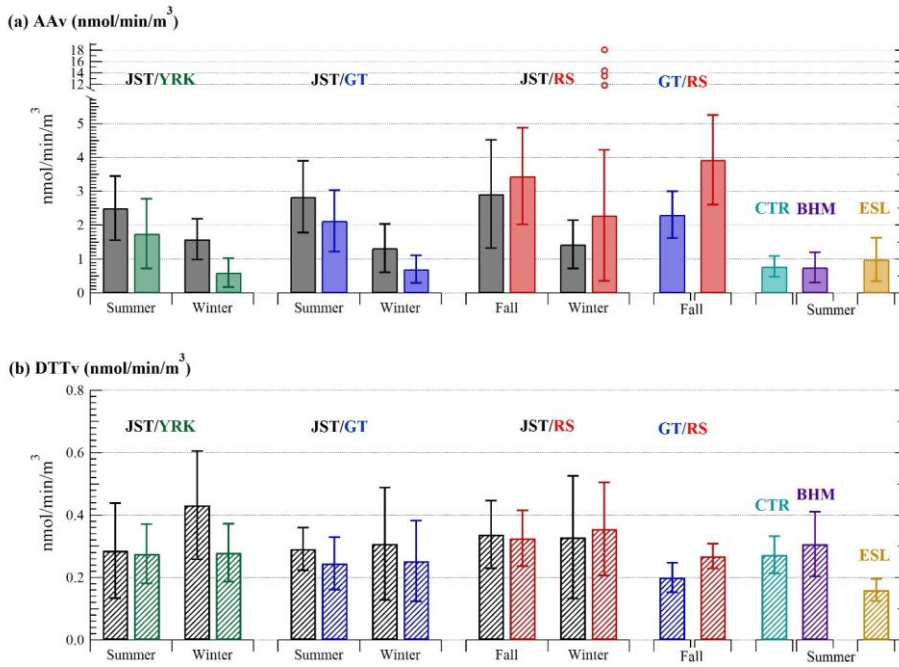


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1725 **Figure 1. Protocol schematics for conducting Ascorbic Acid assay**

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1728 **Figure 12.** Monthly average (\pm SD) of PM_{2.5} oxidative potential based on the (a) AA and (b)
1729 DTT assays from the water-soluble extracts from filters collected at three urban (JST, BHM, and
1730 ESL), two rural (YRK and CTR), a near-road (GT), and a road-side (RS) site in the Southeastern
1731 United States.

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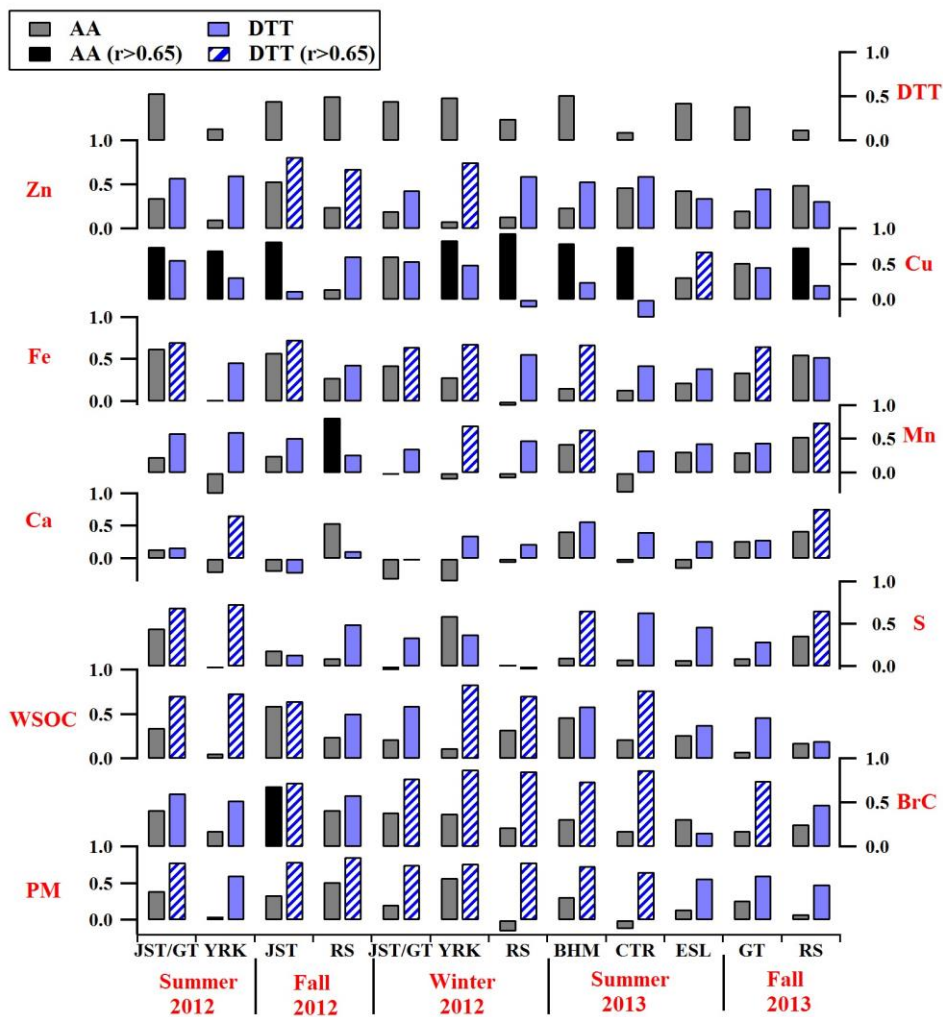
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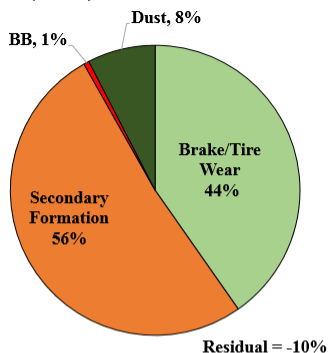
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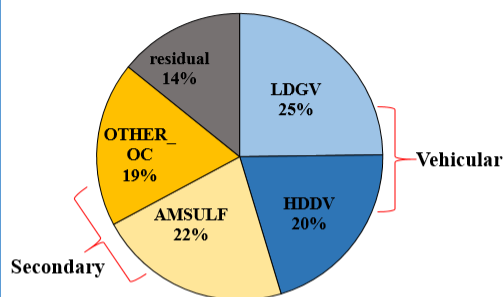
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1739 **Figure 23.** Correlation coefficient (Pearson's r) of fine particle water-soluble AA or DTT
 1740 activities with $PM_{2.5}$ mass and selected chemical species at various sites in the Southeastern US.
 1741 A more detailed correlation table is provided in Table S2.

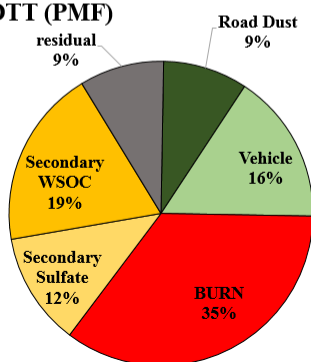
(a) AA (PMF)



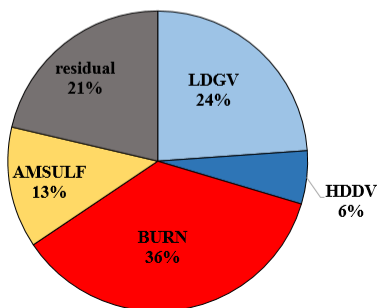
(b) AA (CMB-E)



(c) DTT (PMF)



(d) DTT (CMB-E)



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1743 **Figure 34.** Contribution of various factors resolved by PMF (a, c), and ensemble (b, d), to the
1744 water-soluble AA (a, b) and DTT (c, d) activities measured during 2012-2013. BURN – biomass
1745 burning; AMSULF – ammonium sulfate; HDDV – heavy-duty diesel vehicles; LDGV – light-
1746 duty gasoline vehicles; OTHER_OC – other organic carbon which secondary organic aerosols
1747 from biogenic emissions, and possible additional contributions from other VOC sources contains
1748 mostly biogenic carbons.

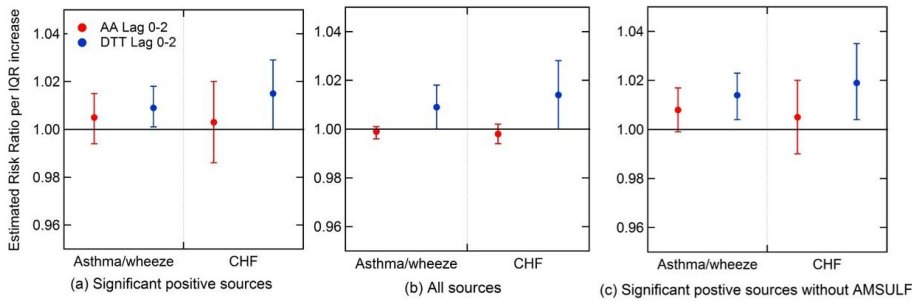
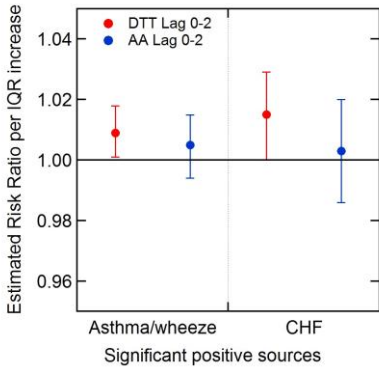


Figure 45. Associations between backcast-estimated AA ~~DTT~~ and DTT ~~AA~~ activities based on estimated sources for the previous 10 years (1998-2009) and emergency department (ED) visits for asthma/wheeze and congestive heart failure (CHF) in the greater metropolitan Atlanta, GA, region. The estimated AA ~~DTT~~ and DTT ~~AA~~ were based on a linear regression models that includes (a) only statistically significant (p of F-statistic of coefficient<0.05) sources with positive coefficients; (b) all sources; and (c) significant positive sources without AMSULF (ammonium sulfate). The models were generated from a multiple regression of the measured AA activities ~~DTT~~ or DTT-AA activities, on a per volume air bases, with all sources from CMB-E as independent variables. Risk ratios and associated 95% confidence intervals are presented for an increase of one interquartile range (IQR) increment of the exposure metric. A risk ratio with

1761 95% confidence intervals (CI) for interquartile range above 1 indicates a statistically significant
1762 positive association. Risk ratio data and related statistics can be found in Table S4. ~~Results from~~
1763 ~~other models are shown in Fig S7.~~
1764