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Interactive comment on "Atmo-metabolomics: a new measurement approach for investigating aerosol composition and ecosystem functioning" *by* Albert Rivas-Ubach et al.

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To editor and reviewers, We thank very much the reviewers for their effort and their time to read our manuscript and provide very valuable comments. After carefully reading all the comments of the two referees, we understand that our manuscript could create confusion. Our main intention was to describe an approach to advance ecological research with all the necessary steps, including sampling, extraction of metabolites in liquid phase, analyses with 3 different instruments, data mining and analysis, to obtain the metabolomic fingerprints from particles in suspension in the lower atmosphere. We expect this method will be useful for addressing novel questions in ecology and other related disciplines. Therefore, it is a methodologic manuscript oriented es-

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Discussion paper

pecially to ecological research and is not intended to target atmospheric chemistry studies, although we recognize that this is more of the focus for the AMT journal. Since this method describes all the details necessary to characterize the metabolomes of aerosols, we thought that AMT was a suitable target journal. However, while we think that our methodology does provide very valuable information for atmospheric scientists, we recognize that this method is aimed mainly to assist the ecological community and now acknowledge that the scope of AMT is more oriented to publish research focused in “advances in remote sensing, as well as in situ and laboratory measurement techniques for the constituents and properties of the Earth’s atmosphere”. After carefully reading all the reviewer comments we believe that our manuscript would probably fit better to an ecological journal but since our manuscript was still considered for revision and will be published in the online discussion, we have now modified the text clarifying the aims of the method. Note that it does address any of the issues related to the field of aerosol chemistry, as this was never our intention. We hope the aims of this methodological manuscript are now clearer.

Anonymous Referee #1 Received and published: 7 October 2016 Referee report for "Atmo-metabolomics: a new measurement approach for investigating aerosol composition and ecosystem functioning” by Albert Rivas-Ubach et al., submitted to AMT This manuscript describes the organic analysis of ambient aerosols with three techniques, GC-MS, LC-MS and direct infusion MS. The focus of this manuscript is not clear at all. The title seems to suggest that a new technique is described but all that is provided are analyses techniques that are used in the community since years. So, I cannot see what the new aspect of this paper is. Creating a new word for existing analysis strategies is not helpful. It is not clear what the focus of the paper should be. The actual results seem to suggest that tracers of PBAP are the main focus but then the manuscript often mentions that the aim is to determine the overall particle composition, which is clearly dominated by many other sources ad not only PBAP.

Response: As mentioned previously, our manuscript is focused on the ecological

community. While these techniques have been available to the scientific community, these metabolomic techniques have not been used to characterize the metabolomes of aerosols. The aim is to provide ecologists and environmental scientists with a tool to assess the ecosystem status and stress levels through the atmospheric detection of biochemical compounds. To demonstrate our method, we collected aerosol samples from two distinct seasons to test our methodology but without attempting a deep analysis of the differences between the seasons. We simply chose seasonality for comparison purposes and to test the sensitivity of the instruments to detect differences between the metabolomic fingerprints; other factors such as different ecosystems could have been chosen too. Metabolomics techniques, which include all the steps from the sampling to the analysis of the data, have been widely used to measure the metabolomes from living systems. However, metabolomics can also be applied to obtain metabolic signatures from any sample contaning natural organic matter (NOM). We acknowledge that diverse mass spectrometry techniques such as GC-MS have been used for years in the atmospheric research, especially to detect and quantify volatile species such as BVOCs. Nonetheless, our purpose in this manuscript was never to improve or replace those well-defined approaches. With the approach that we present in this manuscript, researchers should be able to detect metabolites in aerosols directly linked with the main physiological processes occurring in living organisms. Moreover, our manuscript provides a good synthesis of the main techniques used for metabolomic analyses, including FT-ICR-MS, which we believe it is especially useful for those researchers interested to introduce metabolomics approaches to further understand the link between the atmosphere and ecosystems

Figure 1-3 are to a large extent trivial and would be better suited in a review rather than in a research paper.

Response: The main intention of our manuscript was to explain in detail a methodology of sampling particles in suspension. Figure 1 shows the most common sources of compounds and particulates as well as their major roles and their interaction with

ecosystems, humans and climate. We think it is useful to provide a general background, especially to the readers from ecological and other environmental disciplines, of the main sources and processes of compounds in the atmosphere. Figure 2 describes the sampling method; we consider this to be important for a methodological paper; however, following the referee's comment, we have now moved this figure to the supporting information (Figure S1). This figure provides a general picture of how the sampling was performed and how the cassettes should hold the filters for a homogeneous sampling; important information for researchers that are not familiar with aerosol sampling. Figure 3 (now Figure 2) describes step by step how to extract the metabolites from the filters into a solution. We think showing this figure in the main text of a methodological article for metabolomics analyses is necessary.

p. 4 looks to me more like a conclusion section rather than text for an introduction. The prupose of this section is to make the case for why this approach would be useful for ecologists and other disciplines. It is not based on the results of our study but just shows the need and the potential value of demonstrating this approach.

Response: The results (e.g. in Figure 4-8) show some interesting findings but overall they are hardly discussed and compared to existing, up to date literature. For all the applied techniques (GC, LC, and direct infusion high resolution MS) there are many current publications, which need to be discussed. The main objective of our methodological article is to provide the proof that the "atmo-metabolomes" (metabolomic fingerprints) of spring and summer differ statistically between them. Additionally, the filters were analyzed with three different instruments (GC-MS, LC-MS, FTICR) to test whether they were sensitive enough to detect significant changes ($P < 0.05$) between seasons. To discuss all the details obtained from each instrument would shift the aim of this manuscript and it would considerably lengthen the text. One of the main aims of the study was to provide a method able to discern the differences in aerosol metabolomes between two different seasons with three different instruments. We acknowledge the large aerosol bibliography available using the MS techniques. However, as discussed

above, this article is not intended to be a review of all the bibliography or a revision of current atmospheric sampling techniques. We agree that it is important to show a certain properly referenced background directly related with our instruments and results. In the new version of the manuscript we restructured the introduction providing more information related to MS studies. The space in a journal is limited and the section providing discussion of the results from the three mass spectrometry instruments, which is not the central focus of the article, is already almost 800 words. For this reason, we have not extended this part in order to keep the aim of the study clear in a relatively concise manuscript.

Aerosol sampling (p. 8). It is not clear why the commercial filter holders were modified. This should be clearly motivated.

Response: Filter cassettes do not require modification if they already ensure a homogeneous distribution of the air-flow along the filter surface during the sampling. The commercial cassettes we used in our study were designed in a way that the air did not flow properly along the entire surface of the filters, so they had to be slightly modified to achieve that homogeneous distribution of the air-flow. We wanted to provide all the sampling details in the manuscript but we finally decided to delete those sentences from the M&M section to avoid any confusion.

Filter sampling is used in aerosol sciences since decades and it is a standard method. However, much of the sampling description seems to suggest that a new technique is presented, which is not the case. Aerosol was collected without any upper size cut as it is standard practice in aerosol science. This is a serious short-coming and brings the severe risk that large biological material is collected that would not be transported over significant distances due their large size. Collecting aerosol within a certain size range is absolutely essential for any aerosol sampling and analysis. Therefore, the results of this study cannot claim to represent atmospheric aerosols.

Response: We agree that our sampling procedures do not differ substantially from the

ones used in atmospheric sciences. As a methodological article, its aim is to explain in detail all the main steps in order to obtain the metabolomic fingerprints of the particles in suspension in the low atmosphere with different analytical instruments. In this article we put together in a comprehensive way, especially for the ecological and plant science community, all those steps and we are convinced that a detailed description of our sampling method is necessary. However, contrary to many aerosol sampling methods, our methodology is very flexible, portable and economic, and probably the most important aspect: very simple. As mentioned above, we wanted to put all the details together in a single manuscript and we expect it to be valuable for the research community. Furthermore, as mentioned by the referee, our sampling method can collect large biological material. While an upper cut size can be employed to increase the footprint of the ecosystem represented, the approach used here is suitable for characterizing the ecosystem of the immedidate surrounding area which was our objective for this study. As stated before, the aim of this article was not to provide answers on the chemistry processes occurring in the atmosphere in a specific moment but to explain a method to obtain the metabolomic profiles of the particles in suspension in the atmosphere. For this reason, it is not necessary to sample a specific size range but that sample can include all particles to obtain a general picture of which molecular compounds are present in the particle fraction in the lower atmosphere. We are convinced that this methodological approach provides very valuable information for the ecological community.

GC and LC results. LC results report 18 identified compounds. GC analysis mention 14 compounds. Most comprehensive aerosol analyses presented in the literature using these techniques identify many more compounds. It is not clear why in the study presented here only a small number of compounds was identified. There is no evidence given how these compounds were identified. Simply mentioning “Library identification” is not sufficient. More details would need to be given.

Response: The number of compounds identified and verified in a sample depends

mainly on three factors: i) the solvents used for the extraction of metabolites, ii) the concentration of metabolites in the samples and iii) the specific metabolites present in the metabolite databases. Typically, un-targeted metabolomics techniques have been applied to obtain the metabolic fingerprints and profiles from living organisms. For this reason the metabolite databases include metabolites from living organisms and mainly from their primary metabolism. The focus of our method are compounds known to be metabolites coming from living organisms. According to the metabolite databases used for this study we assigned a bit more than 30 compounds combining both GC and LC-MS methodologies that are directly linked to the metabolism of organisms, likely from plants. Additionally, sampling was performed in an area with very low biological activity compared to more forested areas and organic volatile compounds derived from plants or anthropogenic emissions could not be identified by our libraries since those compounds are simply not normally listed in our metabolomic libraries. As we mentioned in the manuscript: "The techniques to characterize the gas phase component of atmo-metabolomes are well described elsewhere (Smith and Španěl, 2011; Tholl et al., 2006). Our purpose here is to describe an atmo-metabolomic method for sampling aerosols and characterize the particle phase of the atmo-metabolomes.". In the past version of the manuscript we also mentioned that "Metabolite assignation with LC-MS was performed by our metabolite library with more than 200 typical metabolites usually present in plants and fungi including products from primary and secondary metabolism"; so we made it clear which kind of compounds we were targeting. Our intention was never to reproduce a method to sample all atmospheric organic compounds but to measure with LC-MS and GC-MS compounds present in solid particles coming from living systems. We have rewritten the section regarding the metabolite identification by LC-MS. It now reads: "Metabolite assignation with LC-MS was performed by our metabolite library with more than 200 typical metabolites usually present in plants and fungi including products from primary and secondary metabolism. Assignation were performed separately for each ionization mode (positive and negative) and using the exact mass of metabolites and RT. ESI do not typically fragment all

ions, however, in some molecules we could still detect some fragments which were also considered for the metabolite assignation and relative quantification. According to Sumner et al., 2007, our LC-MS metabolite assignment is putative since it was based on total exact mass of the metabolite and RT of standard measurements in the instrument. However, the use of high MS resolution achieved with Orbitrap technology and RT reduces substantially the number of false positive assignations. For more detailed information regarding the metabolite assignation see Rivas-Ubach et al., (2016b). RT and m/z values of metabolite matching for LC-MS are shown in Table S2."

Figure 7 and 8 show interesting results but more discussion would be needed.

Response: Already responded above.

Section 4.1 is mostly trivial discussion and can be shortened a lot. The same applies to much of section 4.3.

Response: Following the referee's advice, we have now shortened the section 4.1 and deleted the section 4.3 re-organizing some content into the introduction.

Anonymous Referee #2 Received and published: 2 October 2016

Overview: This manuscript describes a metabolic-approach for the analysis of atmospheric aerosol. The approach includes GC/MS, LC/MS and direct injection FT-ICRMS measurements. To demonstrate the potential for this method to contribute toward an improved understanding of natural metabolites associated with aerosol, the authors studied the composition of aerosol collected in the spring and the summer. Key results include: the finding that plant-related metabolites (namely organic acids and carbohydrates) are higher in the spring than summer; the summer samples included metabolites associated with oxidative stress; and summer aerosol composition included a higher fraction of high molecular weight compounds than spring with a higher O/C ratio. The manuscript contains very valuable laboratory method information that is well referenced. However, the details about the advanced statistical analysis are deficient. The

introduction and methods sections are well-written, but the results and discussion section seems to be presented poorly. Given the inadequate description of the statistical approach, I found the results section to be especially difficult to understand. Another aspect for further consideration is placing this work into the context of the current literature on aerosol chemistry. There's quite a bit of similar work without a so-called "metabolomics" approach that is relevant.

Response: Many thanks for the positive evaluation on the interest of the study and for considering very valuable the laboratory method information. We have now rewritten the introduction, focusing it on the ecological applications of the study of the metabolomic fingerprint of ecosystems on atmospheric aerosols. And to address the referee's concerns, we have now clarified the statistical methods section and combined the results and discussion. We hope that now the text is clearer. We acknowledge that GC-MS and other mass spectrometry techniques have been widely used in the atmospheric research. Nonetheless, as in our response to the previous referee, our purpose for this manuscript was not to improve or replace those well-defined approaches or to investigate the chemistry of the atmosphere. We present an approach that is novel and useful for the ecological community by enabling researchers to detect aerosol metabolites that may be directly linked with the main physiological and ecological processes of living organisms.

Specific suggestions:

The literature review of atmospheric aerosol composition is weak and outdated. Since the authors claim to be the first to apply metabolomics techniques to aerosol, which are not necessarily different from other composition measurements, it would be nice if they would acknowledge the vast literature of GC/MS, LC/MS and FT-ICR-MS results aimed at understanding aerosol composition.

Response: Our manuscript aims to describe in enough detail the set of necessary procedures to obtain the metabolome profiles from aerosols. As mentioned above, this

method is mainly focused to detect signatures directly linked to the main physiological and ecological processes of organisms; metabolites which are not volatile but are also part of many particles in suspension in the atmosphere. We have modified the introduction more clearly focusing the aims on the ecological aspects.

Lines 102 - 106: How important is the carbon and nutrient deposition of aerosols to ecological systems?

Response: We have expanded the section in the introduction about the aerosol deposition on ecosystems.

Lines 145 - 148: The atmospheric system is quite complex and the goals of this manuscript are quite broad. I suggest some refinement of the manuscript goals with a focus on a well-defined portion of the atmospheric system, since this work doesn't address larger spatial sampling, research flight measurements, or multiphase measurements.

Response: As mentioned above, we have rewritten the introduction section. We have now better focused our manuscript and hope the purposes of our method are clearer.

Line 187: I often see this statement in manuscripts, but it is not a realistic resolving power for environmental samples. Can the authors cite a paper demonstrating the successful measurement of a complex mixture with a resolving power and actual resolution of 1,000,000?

Response: We reviewed the capability of FT-ICR-MS in the manuscript as an introduction of this analytical method. Therefore, we have to report the maximum resolving power that FT-ICR-MS can achieve. However, we did not state that such resolving power is currently used in environmental study. We stated the actual resolving power ($\sim$400,000 at 400 m/z) for our samples.

The organization of sections 2.3 - 2.5 is a little bit strange. Specifically, a description of the GC/MS sample prep (in 2.3) is given followed by LC/MS analysis (2.4), which is in

turn followed by the GC/MS analysis (2.5).

Response: We understand that this may create some confusion, however, we wanted to be consistent and we have followed the same order for the methods and results along the article; LC-MS, GC-MS and FTICR consecutively. The section 2.3 described the extraction of metabolites from the quartz filters which is common for all the three MS techniques (LC, GC and ICR). However, differently to LC-MS and DI-FT-ICR extracts, samples for GC-MS require an additional step; the derivatization of metabolites. This step is also indicated in the Figure 3 and it is clearly linked to the extraction of metabolites. We considered the derivatization should not be in the following section of GC-MS analyses (2.5). However, we could consider moving this section if required. After the section for sample preparation (2.3)(common for the three techniques), we have described the parameters used for each one of the MS instruments separately according to the order established (2.4 for LC-MS, 2.5 for GC-MS and 2.6 for DI-FTICR). Following the instrument analysis sections, the next 3 sections (2.7, 2.8 and 2.9) provide the details to obtain the numerical data from each of the instruments. Also, these 3 sections follow the same order established, so 2.7 for LC-MS, 2.8 for GC-MS and 2.9 for FT-ICR.

So, our logic for the description of the methods was: 1. Extraction of metabolites. (2.3) 2. Data acquisition by each MS instrument (2.4, 2.5 and 2.6) 3. Processing of MS chromatograms/spectra from each instrument. (2.7, 2.8 and 2.9) We think that this order is comprehensive; however, we can change the distribution of the methods if required. An option would be to include all the instruments in a single “Data acquisition” and “Processing of chromatograms/spectra” section by using subtitles.

Line 346: How were both positive and negative ionization performed with LC/MS? Were they done in separate runs or using fast polarity switching? The LTQ Orbitrap Velos cannot switch ionization polarities quickly. Only the most recent Q-Exactive and the new LUMOS Orbitrap versions can operate with fast polarity switch. So samples were first injected in positive mode and then in negative mode. We now have indicated

this detail in the manuscript and it can be read as: “All samples were first analyzed in positive (+) ionization mode and later in negative (-) ionization mode.”

Line 371: Was negative mode ESI performed? Why was negative ESI not performed for atmospheric aerosol characterization?

Response: Analyses in FT-ICR-MS were performed exclusively in negative mode as already mentioned in the manuscript: “Samples were directly infused into the mass spectrometer using a standard Bruker electrospray ionization (ESI) in negative mode at a flow rate of 3.0 μL/min through an Agilent 1200 series pump (Agilent Technologies, Santa Clara, CA, USA .” FT-ICR-MS in negative mode is the most used method to investigate natural organic matter. While positive ESI mode could increase the compound coverage, we opted to use negative mode only as our instrument was optimized under ESI(-) for organic matter exploration.

Lines 381 - 383: Both fragment ions and exact mass were used to assign metabolites. Were these measurements made in single runs LTQ MS/MS and FT-MS in tandem or something else?

Response: Although we already referenced a manuscript where the metabolite assignation is well described, we agree that this section should be more detailed, especially for a methodological article like the present one. We have extended this section of the manuscript. It now reads: “Metabolite assignation with LC-MS was performed by our metabolite library with more than 200 typical metabolites usually present in plants and fungi including products from primary and secondary metabolism. Assignation were performed separately for each ionization mode (positive and negative) and using the exact mass of metabolites and RT. ESI do not typically fragment all ions, however, in some molecules we could still detect some fragments which were also considered for the metabolite assignation and relative quantification. According to Sumner et al., 2007, our LC-MS metabolite assignment is putative since it was based on total exact mass of the metabolite and RT of standard measurements in the instrument. How-

ever, the use of high MS resolution achieved with Orbitrap technology and RT reduces substantially the number of false positive assignations. For more detailed information regarding the metabolite assignation see Rivas-Ubach et al., (2016b). RT and m/z values of metabolite matching for LC-MS are shown in Table S2."

Line 418: Why was S/N > 7 used as a threshold? How was the S/N determined?

Response: S/N was determined in the Bruker Data Analysis software, which was assessed based on baselines near each peak. S/N of 3 and 5 are often used in natural organic matter exploration as that range is considered as the mínimum detection limit (Riedel and Dittmar 2014). We chose S/N>7 for a more conservative measure.

Lines 473 - 476: How many data points were used for this analysis? How were the subsets of data selected for analysis? Some discussion on the QA filtering procedures and selection of data for statistical analysis is greatly needed.

Response: Each analytical technique generated their own data that were posteriorly analyzed separately. All the data (metabolomic fingerprints) from each instrument were used to perform the PERMANOVAs. PERMANOVAs were performed separately. As this manuscript was not especially focused on the understanding of the metabolomes or chemical signatures between summer and spring aerosols, we did not include the number of features we observed and used for the statistical analyses. We can include this information if you think it necessary. We have added some more text in the material and methods section explaining the data filtering in more detail. Now the text reads: "For each season (spring and summer) and dataset (LC-MS, GC-MS and FT-ICR-MS), the variables present in less than 50% of the samples were excluded for the statistical analyses. The signal values measured in the experimental blanks in each of the instruments were subtracted from the datasets. Each of the variables from metabolome fingerprints obtained from each MS instrument were posteriorly submitted to Levene's and Shapiro tests to assess homogeneity of variances and normality, respectively. Variables that did not comply with those statistical assumptions were removed from the

datasets. Outlier measurements were replaced for missing values and were defined as those measurements of a specific variable with values three-fold higher than the third quartile or three-fold lower than the first quartile of each season. For FT-ICR-MS datasets we have been very conservative and only the formula assigned features that presented less than 0.3ppm of error were used although cutoff values up to 0.5ppm are typically used (Osterholz et al., 2016)."

Line 487: In what sense is the statistical significance?

Response: As typically used in the vast majority of environmental studies, the alpha error or type I error is maintained at 5%. The term "statistical significance" is widely used for P values lower than 0.05 for a given test. So, alpha error (type I error), the probability of rejecting the null hypothesis when is true, was maintained at 5%.

Lines 489-496: What do these compounds indicate? How were they identified?

Response: In the results section we only indicate which compounds increased significantly ($P < 0.05$) or marginally significantly ($P < 0.1$) in the spring samples. Some of the metabolites identified are briefly discussed in the discussion section (4.4). We did not discuss all the results obtained with each of the instruments since it would be out of the main aim of the study. This article is just a methodological article and we have focused the discussion on the major results and it was not our intention to investigate all of the differences between the seasons. Those compounds were identified according to our LC-MS database of metabolites of plants and fungi, however, as already mentioned above we have now extended the section of metabolite identification and provided more details.

501-504: This approach from Kim et al. is highly speculative. It's also not an appropriate approach for atmospheric aerosol. Did you extra proteins? How did you verify protein-like components?

Response: We highly agree with the referee. Although the compound classification

obtained from van Krevelen (vK) diagrams (O:C vs. H:C) provides a certain approximation of the composition of the samples, we also think that their use should be limited. However, vK diagrams are widely used to understand the chemical changes in samples and this classification is still widely used to represent the FTICR data. Because this compound classification is a widely used method to understand organic matter composition, our intention was to show this to the readers. However, it should be noted that even in the previous manuscript version we only briefly mentioned this classification. In fact, we are already working on another manuscript reviewing this commonly used compound classification for FTICR data. For this reason, in the new version of the manuscript we finally decided to retain the review of the existence of such classification but we have deleted the previous Figure 7.

Lines 517 - 520: What is the meaning of this observation?

Response: Here we mention that particles in summer showed significantly higher intensities in features with higher O/C ratios. This result is briefly discussed in the discussion method, however, as the aim of the article is solely methodological, we did not discuss each of the results in depth. We simply chose two seasons to test if we could detect statistically significant differences between the "atmo-metabolomes" between the two seasons. Different factors could be chosen for this test, like two different ecosystems but we considered that seasonality was more a feasible and comprehensive factor to test.

Aerosol sampling information is vague and seems to imply that the authors are unfamiliar with standard sampling techniques for atmospheric chemistry. How did you assess the total carbon concentrations, filter artifacts, and other recovery issues?

Response: Our intention was not to reproduce a standard atmospheric chemistry sampling technique as we recognize that there are numerous researchers focused on the chemistry transformations in the atmosphere and for that reason many specific protocols are typically used. However, our simple method is suitable for characterizing the

metabolome of the atmosphere. The aim of metabolomics is to compare relatively different groups of samples. Since it is practically impossible to obtain a full metabolome in terms of absolute concentrations for each of the detected metabolites, as long as the sample preparation is performed equally for all the samples we can perform a relative comparison between groups of samples. Filter artifacts were coped with experimental blanks that were injected to all instruments and any signal obtained from those blanks was posteriorly subtracted from the original samples. The use of blanks is a standard procedure for any metabolomics study. We have now included more details in the material and methods to respond to those concerns.

Sampling flow rates are expected to change with diurnal cycles (e.g., temperature & pressure); how was this recorded or accounted for?

Response: Each filter was sampled exactly for the same amount of time and in the same time range as described in the material and methods section: "The pump was working daily during 18 consecutive hours and pumped air at 30 L per minute through each filter. Filters were replaced manually before 09:00am and the pump started working automatically at 09:00am and stopped automatically at 03:00am the following day. Filters were stored at -80átŠC until metabolite extraction. Filters were sampled on a tower at 8 meters height."

Lines 535 - 537: The purpose of the study was to assess the sensitivity of different mass spectrometry instruments. But, I didn't understand how that was accomplished? Did you define method detection limits or find any limitations in your approach? More discussion on this would be appreciated.

Response: We rely on the statistical analyses to test the sensitivity of the used techniques to detect changes between seasons. We sampled in an area with a very low primary producer activity and still we were able to detect significant differences in the overall atmo-metabolomes between spring and summer. The significance obtained in the PERMANOVA test proves that each of the techniques was sensitive enough to de-

tect changes between those samples. The principal component analyses (PCAs) for each of the instruments also prove that the instruments were able to detect significantly different overall composition in the spring vs. summer samples. In order to clarify this concern, we have modified the text properly in different sections.

How does you approach differ from the existing approaches to canopy measurements or other ecological studies focused on atmospheric-biosphere exchange?

Response: In this article we explained, and put together, the different steps to obtain the metabolomic fingerpints (or metabolomic signatures) from particles sampled in the lower atmosphere. As far as we know, no other approach for analyzing aerosol metabolomes has been published. Similar sampling methods can be performed in other ways with different pumps and filters, however, the method we propose is more portable (lower weight and volume), flexible (can be easily manipulated in different ways) and more economic than the commonly commercialized prototypes for aerosol filter sampling. Also as a methodological article we provided detailed information on how our sampling was designed and performed. As discussed in the manuscript, the main idea is to obtain the minimum values in the filter-size/pump-flow ratio to concentrate as much as possible the filters. Our objective was not to perform a comparative study with all the available sampling methods. We just described a very simple and flexible method that samples particles in suspension efficiently and at a low cost. In addition, researchers can choose the filter size they require while many commercial systems are compatible only with a unique filter size.

Lines 584-587: Which solvents did you use to sequentially extract the filters? How did you evaluate the results of various solvent combinations?

Response: We did not perform a sequential extraction in this study. We used methanol:water (80:20) as one of the most widely used solvent mix for extraction of metabolites. We cite different studies and methods where the number of extractions and recovery is discussed. We did not attempt to use a whole variety of extraction

methods; we only aimed to show a generally used extraction method to investigate whether the analytical techniques can differenciate statistically the metabolomes between spring and summer aerosols. We also mention that this extraction method is not exclusive but suggested and indicate that different extraction methods can be also used. As widely discussed in several analytical chemistry articles, different extraction methods obtain different range of metabolites based mainly on their polarity.

Lines 590-591: What was quantified in your study?

Response: In this study we performed a relative quantification of the metabolomic fingerprints for comparative analyses between spring and summer.

Lines 596 - 600: How was the absorption extract recovery assessed?

Response: We measured how much volume of solvent was recovered (after the extraction procedures) with respect to the initial solvent added. In the text we mention that we can get an extraction recovery of 89% which indicates that we recover 0.89mL per each 1mL added to the tubes with the filters to perform the extraction. We did not think that it was necessary to incorporate this information in the methods section. However, we can introduce the explanation if the referee thinks it is necessary.

Line 623: "match" or assign?

Response: We appreciate you made us notice this, we agree “assign” is more suitable than “match” in this sentence. We have changed the word in the manuscript.

Lines 706-710: Please clarify how the "metabolic fingerprint" was defined/classified?

Response: We have added a clarification of metabolomic fingerprints and metabolomic profiles in the introduction section. Now the text reads: “The first step to characterize a metabolome profile is to obtain the chemical signature of the sample (metabolomic fingerprint) without further molecular identification (Sardans et al., 2011). The identification of specific metabolites can be further obtained by the information present in the metabolomic fingerprints. In this study, we describe the different procedures to obtain

the metabolomic fingerprints and identify molecular compounds from aerosols. This atmo-ecometabolomics methodology is a potential tool to shed light in novel questions in ecology, especially for the ecosystem-atmosphere interface."

Table 1: Fingerprint information is unclear. Please add some explanation in the body of the paper.

Respone:See comment above.

Figure 1: What about aqueous phase processing of VOCs or aerosol?

Response: We modified the figure but the different atmospheric VOCs transformations are not presented in detail since it was never our intention to address that issue in this manuscript.

Figure 3: How were common inorganic ions removed from the samples before DI-FT-ICR-MS?

Response: It should be noted that most of the inorganic ions are at much lower mass range than our FTICR-MS analytical window (100-1200 m/z). Thus, unless those ions generate clusters that would interfere with the FT-ICR-MS measurements, such as sodium and chloride, removal of inorganic ions were not necessary. In addition, such a problem is more evident in direct infusion positive ion mode, which was not considered in this study.

Figure 5: I assume this is the list of "metabolic fingerprint" species. Please clarify.

Response: As mentioned before we have included the definition of what a metabolic fingerprint is. The list of metabolites does not represent the entire fingerprint of the different seasons but only the portion that has been identified/assigned. We hope it is now clearer.

Figure 7: How were the species in (a) subsetted from the whole dataset?

Response: As explained before, we did not use a subset of the datasets but the whole

amount of detected features. However, we have now deleted this figure from the new manuscript version.

Please also note the supplement to this comment:
http://www.atmos-meas-tech-discuss.net/amt-2016-209/amt-2016-209-AC1-supplement.pdf

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Interactive comment on Atmos. Meas. Tech. Discuss., doi:10.5194/amt-2016-209, 2016.

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Discussion paper