

Interactive comment on “Volatile Organic Compound emissions from soil: using Proton-Transfer-Reaction Time-of-Flight Mass Spectrometry (PTR-TOF-MS) for the real time observation of microbial processes” by P. R. Veres et al.

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Anonymous Referee #1 Comment

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General comments

The paper presents an interesting method and shows some potential of combined soil VOC and N trace gas measurements, but it is premature and needs more elaboration on the basis of soils from a broader range of ecosystems, as well as an unambiguous elucidation of the processes responsible for the observed VOC and NO emissions. Although the authors claim that “These experiments can be used as a template for future experiments to more completely and specifically identify the active microbial guilds in soils and to characterize the impact of soil VOC emissions on the atmosphere” (last sentence of the abstract) and by this try to justify a publication of their very preliminary results, still a minimum requirement would have been to show that the VOC emission pattern observed for the arid soil can be considered as a kind of general feature. As there is no comparable information for other soils, even not for the second soil of the study, it remains completely unclear whether we deal here with a new, elegant method for detecting soil microbial activity, or whether we just see an arbitrary snapshot of a laboratory experiment which has nothing to do with natural conditions.

Response: We are pleased that the reviewer has recognized the exciting potential of combined VOC and NO trace gas measurements for soil analysis through the results presented in this paper. However, the reviewer has expressed concern that this study may be premature and has raised the specific point that our study focuses too much on a single soil. Our study focuses on two contrasting soils in order to demonstrate the power of this new combined analytical technique approach and the chemical detail revealed in the incubation/drying out experiments. We agree with the reviewer that we have focused too much on one of the soils and have accordingly extended the VOC:NO analysis to the other soil as well (see additional figures 5 and accompanying text). We do not believe the study to be premature since the equipment used has been in development for 15 years. A comprehensive worldwide soil assessment for this new combination of methods is beyond the scope of the first paper specifically. This work has been designed to demonstrate how the entirely novel combination of VOC

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and NO can be exploited in the future to assess and elucidate microbial soil emissions. Additionally, the manuscript has been edited to place more emphasis on the techniques and methodology. The next step will be to apply this new approach to a larger dataset of globally relevant soil types. We strongly believe the novelty of the VOC/NO linkages and water/temperature dependencies of the emissions merit publication as the first step in this process

In particular, the paper suffers from the following points:

1. No clear mechanistic relationship between the VOC presented and soil microbiological activity has been elaborated. It remains elusive and speculative from which process(es) the VOC originate, and whether the VOC originate from the same microbiological process(es) as NO. It even remains unclear whether the VOC and the NO originate from biological processes at all, as Q10 values of 2-3 are not exclusively indicative of biological processes. Many chemical reactions have Q10 values of 2-4 over a broad temperature range. Therefore, any conclusions pertaining to the suitability of VOC for identification of soil microbiological processes are based on too weak a ground.

Response: We have argued based on an atmospheric chemistry perspective, according to the works of Skopp et al. (1990) and Placella et al. (2012/2013), that a simultaneous release of certain VOC and NO with an optimum shape function over the drying out period is a clear indication that microbiological processes in addition to physicochemical processes might be involved. Especially for very reactive VOC it is very challenging to separate these processes. We prefer arguing based on the position presently expressed in the peer reviewed literature, since any kind of sterilization of soil will only reduce the total number of microbes several orders of magnitude, but additionally change its chemistry (nutrient status, chemical binding sites, etc., see Insam and Seewald, 2010) completely. The result - a sterilized soil - is an artificial (caused by the sterilization) chemical source of VOC and in addition an increasing source of VOC caused by a recovering microbial community over the days of the drying out experi-

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ments. We agree with the referee, that such laboratory experiments are an arbitrary snapshot with limited relation to natural conditions since at each location environmental parameters such as rain, sun, temperature etc will vary considerably. Therefore, we recommend the simultaneous usage of molecular tools in future studies, but this is beyond the scope of this study.

2. The authors present the concept of different soil microbiological "emitting guilds", but it is developed only on data of one soil (arid soil), measured only once during one rewetting-drying period. There is no evidence that the observed pattern would occur again after the second or further rewetting-drying cycles. For the second soil, no such pattern is presented. And as there are no data for other soils, it remains purely speculative whether this is a general feature, only specific for the analyzed soil, an experimental artifact, or perhaps pure coincidence.

Response: We acknowledge that only presenting NO:VOC results for a single soil sample was limiting and have therefore expanded the analysis to the second soil sample as additional evidence of the validity of this technique. As such we have added a 5th figure (similar to fig. 4) containing that analysis as well as a substantial description of the results in the text. Performing several re-wetting and drying out cycles with one soil sample would not be insightful with regard to reproducibility due to the limitation of substrate (C and N), so that it would not be surprising to observe decreasing release rates of VOC with subsequent experiments. Additionally the time for surface adsorption of VOC will be short such that this desorption effect observed upon wetting is expected to be limited. This would be a key result as in these experiments the magnitude of that desorption effect was found to dominate over microbial production processes for VOC.

3. The relevance of the findings is strongly relativized by the authors themselves ("The extent to which these VOC emissions will reach the atmosphere will depend on concomitant uptake processes in the more complex soil ecosystems found in nature", p. 12022, l. 4-5). Therefore, the applicability of the laboratory method demonstrated here to the "real world" (i.e., field campaign measurements on intact soil surfaces), is ques-

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tionable. Despite the uncertainty of the relevance of the work for field conditions, the experimental data could in general still be interesting for getting a closer mechanistic insight into the role of soil microorganisms in soil VOC emissions and their relationship with soil NO emissions. However, also for this purpose the experimental design is insufficient and suffers from serious flaws due to the following reasons:

Response: Many peer-reviewed publications, many of which are cited throughout the introduction, discuss the representativeness of laboratory NO emission studies as a model for “real world” systems. Of course, similar to those previous studies, we have to relativize our findings, since, by design we have no information about the release (production and consumption of VOC) in deeper layers of soil. We are not intending to state that these techniques can be directly extrapolated to describe emissions from soils in the “real world”. This work purposefully avoids placing these results in context of in-situ measurements. In fact, that is why we have made statements such as the above quoted line, p. 12022, l. 4-5. This work is intended to show the power of coupling these analytical techniques to probe soils on the microbial level in the laboratory. Therefore we feel that it is not reasonable to criticize this work based on the difference between field-measured samples and laboratory processed samples. Additionally, in the field, there exist many parameters that are constantly in flux complicating the system making an analysis such as that presented here difficult to interpret. The reviewer does comment exactly on the target of this work in the above critique however “the experimental data could in general still be interesting for getting a closer mechanistic insight into the role of soil microorganisms in soil VOC emissions and their relationship with soil NO emissions.” We are indeed presenting novel methods for breaking down the complexity of soil ecosystems in order to better understand the processes that contribute to emissions of soil VOC. This work is not the terminus of experiments on VOC-microbial relationships but we do not believe that there are serious flaws that would prohibit publications of these new analytical methods.

Below we will attempt to address all of the reviewers concerns.

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1. Only two different soils were used, of which one (rainforest soil) was measured fieldmoist “immediately upon receipt”, whereas the other (arid soil) was air-dried, stored at 4 °C for and rewetted before analysis.

Response: Indeed the soil samples used were quite different and the reasons for these choices are described in the first paragraph in section 2. We used largely contrasting soils to show the variations in the results obtained, but also the similarities in the observations garnered from use of these new methods presented in this work. The differences in storage and sampling is unfortunately not ideal, however impossible to avoid as it was not feasible to sample fresh soil samples from widely varying global locations. We do in fact reference many works showing/discussing the various issues with the different types of sampling and therefore believe that we are not misrepresenting the data here.

2. Only one sample per soil was measured, and each sample was measured only once. At least data of only one measurement each are presented without any information about variability.

Response: Previous publications have shown repeatedly that laboratory soil experiments by nature are highly variable from sample to sample and run to run. We are not attempting to quantify emissions for a particular VOC from a specific microbial group or even ecosystem here and therefore do not feel the need to show multiple runs from a single soil to ensure representativeness for a soil region. Rather we are focused on the information contained in a given experiment and how it can be used to focus research on microbial populations in soils. We have attempted to better enunciate this point in added discussions in section 3.3 and the conclusions section where we openly discuss the short falls of this study and the targets for future work.

3. No nitric oxide (NO) data are presented for the rainforest soil.

Response: We agree with the reviewer that addition of NO will benefit the reader and therefore have added NO to figure 3.

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4. There is a data gap between 30-50 h for the rainforest soil. To summarize, the design of the study needs substantial improvements. The authors make similar suggestions in the discussion section (e.g., p. 12022, l. 13-18: "Future experiments to determine the temperature optimum of VOC emissions and microbial community activity can be pursued as further evidence of biological production: : Furthermore, similar measurements using pure cultures of the active microbiological constituents could help to identify and validate a biological source of soil VOC."; and p. 12024, l. 2-3: "Sub-samples of soil during peak activity periods have to be subjected to molecular analysis in order to better attribute these microbial processes to the release C5185of VOC").

Response: We agree with the reviewer that the gap in the measurement is a negative feature of the experiment. As such, we have made the decision to remove the data collected after the gap and choose to focus on the information contained within the first half of that particular experiment. Experimental issues, such as an instrument failure in this case, are a frequent occurrence in laboratory experiments; however, we feel that the gap does not detract from the conclusions that we are drawing from these experiments. The suggestions that the reviewer is making with respect to our own acknowledgement that these are very new experiments is correct, the design can be improved. We feel, however, that due to the unique nature of these results and limited amount of soil VOC research this work merits publication as a starting point and guide for future experiments. The quoted suggestions in the above comment, made from our own acknowledgements of the shortfalls of this work, constitutes years of research over many disciplines using many different technologies, and is far beyond what could be accomplished in a single manuscript. After considering the reviewers concerns carefully we feel that the idea that the experiments presented here are incomplete without including results from those additional research items is unreasonable.

Specific comments

p. 12013, l. 5-6: The statement "since abiotic and biotic processes should exhibit a different response to temperature" only holds true for the same reaction which could

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proceed either purely chemically or also biologically (enzymatically catalyzed). Then one can determine the activation energy, compare it with chemical data tables or textbooks, and then decide whether the observed reaction is a purely chemical or biological process.

Response: We thank the reviewer for this comment; however as we are not attempting to definitively define the origin of the VOC observed, merely that these new powerful methods could yield additional information on soil VOC emissions, we do not feel any change to the manuscript, with respect to the above comment, is necessary.

p. 12012, l. 6ff.: Give examples of such "unspecific" reactions. Not every reader will be familiar with the enzymes involved in NO production in the soil, and very likely even fewer with the kind of "side reactions" these enzymes could undergo in terms of VOC turnover. Thus, name examples of VOC classes that could be involved. This will be crucial for the interpretation of the data presented, i.e. whether it is likely that the VOC data shown could be a result of microbial activity related to NO production in the soil.

Response: We have now included many references in the introduction to which the reader can be directed for additional information on unspecific enzymatic reactions in soils, also section 3.1 "As mentioned previously, there is evidence that nonspecific enzymes, e.g. ammonium monooxygenase (AMO), can produce various VOC in soil (Arp and Stein, 2003; Keener and Arp, 1994;Hymann et al. 1988)." Rather than detail the origin of VOC definitively, in this work we are focusing on the new techniques presented here for observations of soil VOC emissions and possible relationships to NO. Because of the limited data available we wish not to elaborate on the specific origin of VOC observed, rather we focus on the information that can be garnered from the experimental framework presented here. In the manuscript we make it clear that without combining this method with additional techniques the origin of the VOC cannot be definitively identified.

p. 12015, l. 6-8: The difference in sample treatment is highly problematic with respect

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to microbial activity, as can be seen from the very paper cited here (Stotzky et al., 1962), where it is clearly stated that air-drying leads to a significant decline in microbial activity and changes microbial species composition (as does storage of field-moist soil in closed sample bags).

Response: We acknowledge that the microbial content of the soil sample is potentially altered by the storage methods. This is a real world problem common to all analyses of this kind where laboratory investigation of responses to specific parameters is performed on environmental samples acquired globally. However, our intent is not to present a comprehensive study attributing the observations of VOC to microbial processes and extrapolation to real world systems. Rather, the focus of this paper is to present an innovative combination of analytical techniques that shows promise in retrieving improved, relative to previous methods, information about microbial populations and processes. Our pretreatment methods were applied to reflect, as closely as possible, the natural conditions of the soils, considering the difficulties of laboratory analysis of soil samples.

p. 12017, l. 25: NO is, unlike CO₂, CH₄ and N₂O, not a primary greenhouse gas, but contributes to global warming through tropospheric ozone formation.

Response: The reviewer is correct, and we acknowledge that semantically we are incorrect in grouping NO with those other direct greenhouse gases. The mention of NO has been removed from that line in the manuscript.

p. 12018, l. 1-2: Using clean, VOC-free zero air can lead to increased VOC release from any source (soil, leaves: : :) by shifting the equilibrium between release and uptake completely to the side of release. This artificial experimental effect should be taken into account and discussed.

Response: For clarification page 12018, line 2 has been expanded to read "In this study we use clean, zero air to flush the soil chambers thereby focusing solely on the emission of VOC. We acknowledge that this does not necessarily represent a field

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relevant situation; however, this work is focused on the information about the microbial processes and content of the soils contained in the concomitant release of VOC and NO." We firmly believe that this paper illustrates the value of using clean VOC-free zero air allowing one to focus on the processes occurring within the soil system that contribute to VOC emission, not necessarily as a tool for understanding the quantitative contribution of soils. These VOC observations allow for a more targeted approach to probing the soil system processes beyond those previously published which largely focus on CO₂, NO, and various greenhouse gases.

p. 12018, l. 18: "the mechanisms described in the introduction": There are no mechanisms described in the introduction. It is only mentioned there that "the enzymes responsible for soil emissions of NO are unspecific and thereby can react with various volatile organic compounds (VOC)", but this statement is not followed by a description of the mechanisms, which would be very helpful.

Response: On page 12013, lines 1-5 we discuss several mechanisms that could lead to the abiotic release of VOC. This sentence has been improved for clarity and now reads: "...either rapidly responding microbial activity (Placella et al., 2012), or abiotic processes such as (i) efflux of intracellular solutes (Kleff et al., 1987), (ii) extracellular enzymes from dead microbes (Blagodatskava and Kuzyakov, 2013), or (iii) chemidesorption from the soil surface (Warneke et al., 1999)." Where this list and accompanying references details the abiotic mechanisms referred to in the text.

p. 12020, l. 2-3: Which reaction is meant here? Reaction of NH₄⁺ with hydroxylamine? NH₄⁺ has the oxidation number -3, hydroxylamine has the oxidation number -1. It is not clear how the two should react to NO with the oxidation number +2 without further oxidant. Please explain.

Response: For additional information we point the reviewer to the following:

Daniel J. Arp and Lisa Y. Stein, *Critical Reviews in Biochemistry and Molecular Biology*, 38:471–495, 2003, DOI: 10.1080/10409230390267446 *Metabolism of Inorganic*

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N Compounds by Ammonia-Oxidizing Bacteria

It is not NH_4^+ and instead NH_3 which enzymatically reacts to NH_2OH . See page 473: "First, nitric and nitrous oxide are byproducts of the incomplete oxidation of hydroxylamine to nitrite by HAO, which is seen in cell extracts and may also occur in intact cells. Second, both N oxides can be formed by reduction of nitrite in the process of nitrifier denitrification." For more detailed reaction kinetics, the general textbook (Nitrification, 2011, Ward, Daniel J. Arp, Martin G. Klotz) is recommended.

To clarify/correct in the text, we have edited this portion of the manuscript to read "In the first interpretation, nonspecific enzymes will convert pre-cursor organics rather than NH_3 (Arp and Stein, 2003) to lighter weight VOC that will be released into the gas phase from the soil. NH_4^+ will therefore not be available for reaction to hydroxylamine thereby inhibiting the formation of NO."

p. 12020, l. 3-5: "It is likely that in addition to AMO, there exist other enzymes that are also nonspecific resulting in further reduction of the release rate of NO." This statement needs more detailing, especially pertaining to potential suppression mechanisms of NO production.

Response: While we do believe that the above statement is likely true, we have no hard evidence of such a claim and have therefore removed this sentence from the manuscript.

p. 12020, l. 5-6: Again, it is not clear why a "high mass-loading of organics" should inhibit NO production.

Response: Additional information on this can found in detail in Arp and Stein 2003. Briefly, VOC act in place of NH_3 as a substrate for AMO and therefore AMO will not produce Hydroxylamine, so NO release should decrease.

p. 12021, l. 3: Also many purely chemical reactions have a Q10 value of 2-3.

Response: We do not deny that there may exist chemical reactions that result in a Q10-
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value of 2-3; however, the Q10-value has been widely used in a plethora of published works as an indication of biological activity. Due to the unavailability of other techniques able to determine biological origin, the Q10 metric was our only means necessary for identifying potential biological emissions. This is one of the reasons we suggest that the new techniques presented here need to be applied in the future with more advanced methods to further investigate the linkage between observed emissions any microbial activity, again methods that were unavailable during this work.

p. 12021, l. 20-22: Again, Q10 values of 2-3 are not exclusively indicative of biological activity. A clear proof of biological activity would be an emission maximum at, e.g., 40_C with subsequent decline at higher temperatures due to degeneration of enzymes. Almost every chemical reaction features an increase in reaction velocity by a factor of 2-4 when the temperature is increased by 10_C.

Response: Again we understand that in this work we are only able to speculate as to the sources of VOC as no techniques were applied to definitively determine biologic origin. However, we are focusing on the benefits of the PTR-TOF-MS technique and the potential relationship of the VOC to NO. Discussion of VOC origin and potential microbial relationship is used to place the results of these experiments in context.

p. 12022, l. 13-18: That's the way to go and would have been needed to address the objectives of this paper, i.e., assessing the suitability of VOC as indicators of soil microbiological activity.

Response: We agree with the review that that experimental scenario is indeed the way to go, which is why we ourselves have suggested it. However, we hope the reviewer will agree that the types of experiments suggested there would take a considerable effort of many scientists over a long time period to complete. We believe our work presents a range of results that can be obtained by applying VOC measurements using this new PTR-TOF-MS instrument in a unique manner, and that these methods are very complimentary to traditional methods of soil system analysis.

Technical corrections

Response: All of the following technical corrections have been addressed in the edited text for resubmission.

p. 12011, l. 18: Change “Xinijang” to “Xinjiang”

p. 12013, l. 22: Change “are” to “be”.

p. 12013, l. 29 and p. 12016, l. 3: Change “are” to “were”.

p. 12014, l. 6-7: Change “occurring with” to “occurring in”

p. 12014, l. 25: Delete “–” before “55.” (already indicated by “W”).

p. 12018, l. 23: “occurs”: here and in the following, use past tense for the description of your results.

p. 12019, l. 29: Change “pre-cursor” to “precursor”.

p. 12021, l. 6/7: Change “a temperatures of T1 and T2 respectively” to “at temperatures T1 and T2, respectively”

Interactive comment on Biogeosciences Discuss., 11, 12009, 2014.

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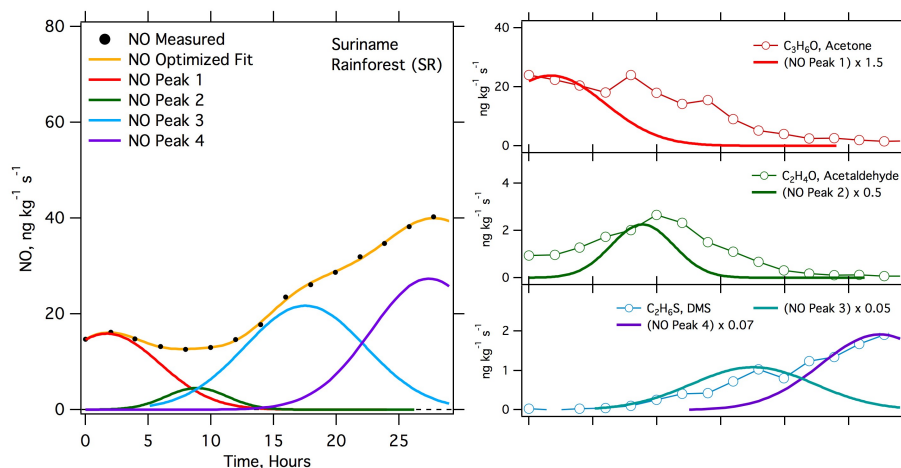


Fig. 1. Figure 5 addition

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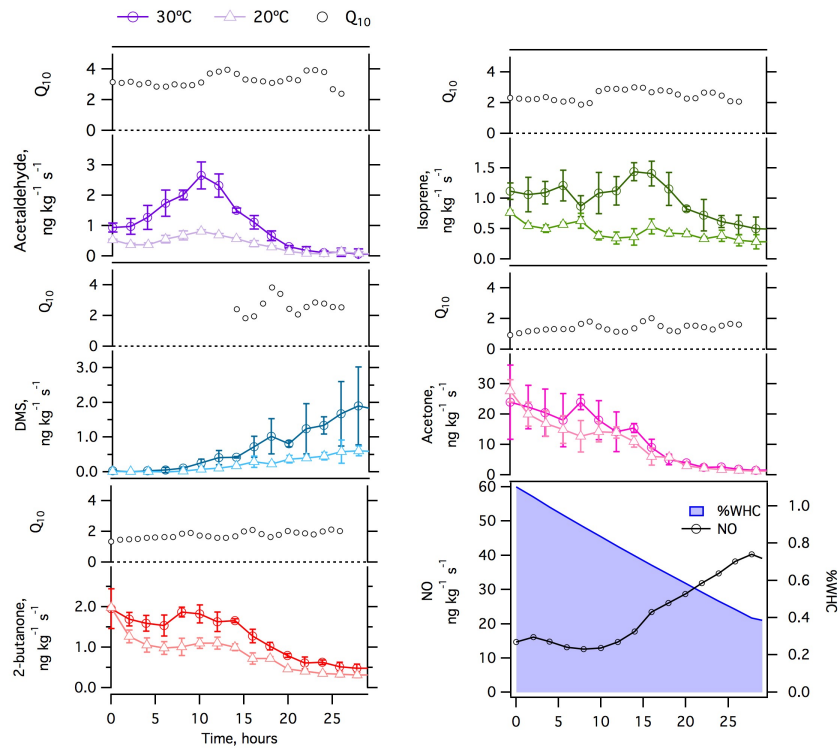


Fig. 2. Figure 3 edited

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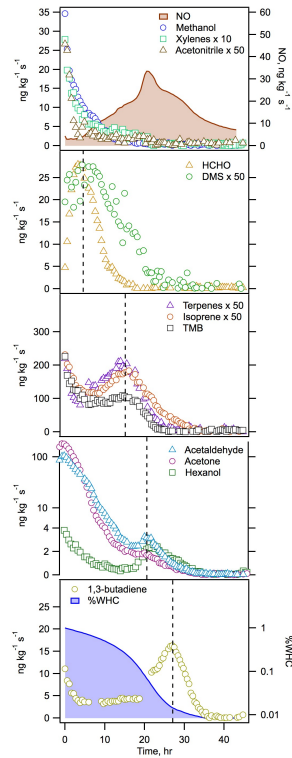


Fig. 3. Figure 2 corrected

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