Interactive comment on

"Emission of nitrous acid from soil and biological soil crusts represents a dominant source of HONO in the remote atmosphere in Cyprus"

by Hannah Meusel et al.

Anonymous Referee #2

Overview:
The stated objective of this study is to characterize and quantify direct emissions of HONO and NO from soil samples collected from Cyprus. This is a follow-up paper to a study by the same group aimed at characterizing daytime formation of HONO during a larger field campaign (CYPHEX, summer 2014) in the same region of Cyprus. That study concluded that soil microbial source of HONO and NO may have contributed the measured mixing ratios of these gases. The present manuscript seeks to make that connection between those emissions and soil by carrying out chamber studies on soil collected at this site. The study site was characterized qualitatively using a gridded transect and visual identification to categorize nine types of ground cover (bare soil, light and dark cyanobacteria, chlorolichen, cyanolichen, moss-dominated, stone, litter, and vascular vegetation/shrub). Six of these soil coverage types were sampled and transported to lab to measure HONO and NO emissions using a dynamic chamber method. In addition, the chlorophyll and nutrient (ammonium, nitrate, and nitrite) levels of those samples were measured. Fluxes of gaseous HONO (measured via LOPAP) and NO (measured by chemluminescence) were found to be highest for bare soil, followed by light and dark biocrusts (Light and Dark BSC), which comprise a combined 2.5, 10, and 6 % of the total ground coverage, respectively. Emissions of HONO and NO were correlated to soil nitrite and nitrate levels (not ammonium or other parameters measured). Flux data along with surface coverage information was used to scale up fluxes in an attempt to estimate the contribution of biogenic soil emissions to the HONO and NO budget determined for the CYPHEX campaign. The conclusion of the paper is that biocrust emissions may close the Cyprus HONO budget.

The paper is clear, statistical methods are appropriate and the topic is of interest to the atmospheric science and biogeochemistry communities.
I have the following concerns about this manuscript regarding the study's approach, the appropriateness of the laboratory flux approach, and its conclusions.

Comment:
***Sampling methods**. Section 2.1 on sampling methods focuses on the procedure used to visually assign and quantify the surface coverage using the grid method, but lacks details on the sampling method used to collect samples for the laboratory chamber study. Details are limited to: "Each sample was collected in a plastic petri dish, sealed and stored in the dark at room temperature until further analysis (storage time less than 15 weeks)." What form did these samples have? What was their dimension and mass? How deep did the samples extend into the ground? Was the sample that was placed into the soil chamber a whole core or was it sieved and/or prepared in any way? The authors state that the storage time in laboratory was less than 15 weeks. Were samples around this long before the nutrient levels were measured, or were nutrient measurements made sooner. Much can happen 15 weeks, and nitrification can be taking place during storage that changes the nutrient pool and impact the lab measurements. This can contribute to significant variability of certain soil measurements.*

Response:
In order to take a sample in the field, the bottom part of a plastic petridish (diameter: 5.5 cm, height: 1 cm) was place upside down on the soil surface and pressed into the soil. A trowel was pushed below the base and together with the samples it was lifted from the ground and carefully turned around to remove surplus soil. The sample was closed with the upper lid of the perti dish, sealed with parafilm and tape and labelled. All samples were taken in dry state. If wet samples had been taken, these would have needed to be fully dried before sealing. The mass of those samples ranged between 25 and 35 g. The biocrust samples consist of a few mm of biocrust and

the underlying soil (total height about 1 cm). For the chamber measurements the whole samples were used, so that the biocrust was intact/undamaged. The samples were measured in an untreated manner and only the samples for the nutrient and chlorophyll analysis were ground.
The storage (time) has a no significant impact on nutrient content and hence HONO and NO emissions, as co-authors of this study have investigated recently (see also comment to reviewer #1). Great care was taken that the biocrusts were stored in a dry state and in the dark to make sure that they are inactive.
The revised manuscript states:
Chapter 2.1: "Each sample was collected in dry state in a plastic petri dish (diameter 5.5. cm, height 1 cm), sealed and stored in the dark at room temperature until further analysis (storage time less than 15 weeks). Based on previous experiments in our laboratory, it can be anticipated that the sample's chemical (nutrient content) and biological (chlorophyll content) properties were not deteriorated during storage (a manuscript on this study will be submitted soon)."
Chapter 2.4: "Intact soil and biocrust samples (25-35 g in a plastic petri dish with 5.5 cm diameter and about 1 cm height) were wetted with 8-13 g of pure water (18.2 MΩ) up to full water holding capacity and placed into a dynamic Teflon film chamber…Intact (biocrust) samples consist of a few mm of the biocrust and the underlying soil."
In the original manuscript (chapter 2.3) it was already stated, that the samples were ground for nutrient and chlorophyll analysis ("…the samples comprised of soil and its biocrust-cover were gently ground…", "Ground samples were extracted twice…")

Comment:
The sampling procedure and consistency/physical properties of the sample that was placed in the chambers is critical for this type of study. There has been a debate among researchers about how representative gas fluxes are for sieved or cored soil samples of environmental conditions. Previous studies suggest that such laboratory studies of soil cores give similar flux measurements as eddy covariance for grassland soils. In such soils, the surface porosity can be considered to be more similar to porosity of soil just below the surface and arguments could be made that gas exchange from soil in the field and in laboratory cores might be similar. However, biocrusts may present a particularly difficult biome to sample in this way since the intact soil and disturbed soil may have very different structural properties. The physical structure of these surfaces is defined by a network of filamentous growth and biomass that creates a hard crust that is often an impermeable layer that may impact gas exchange. These structural features are known to form hard crusts that prevent soil erosion in sensitive arid ecosystems. The soil exposed when soil is extracted as a core or sieved soil may provide a means to bypass surface structural properties that hinder gas exchange. Do the authors have any evidence to suggest that their method of sampling did not impact gas exchange from these samples? It is important to demonstrate that the results are close to reality and can be used for the type of scaled up estimation performed at the end of the manuscript.

Response:
In order to study the emissions from biocrusts, the samples must be intact as sieving would destroy the crust network/community which probably has an impact on exchange processes. To be as much representative as possible, we made sure that the whole core samples were not sieved or otherwise modified. Although the crust surface, especially with cyanobacteria, is quiet hard, it allows for exchange of nitrogen gases (please see Weber et al., 2015). Earlier studies have shown that NO emissions obtained by the dynamic chamber are consistent with flux measurements in the field (van Dijk et al., 2002; Rummel et al., 2002).
Added to the manuscript in chapter 2.4 (page 4, lines 34-35): "The dynamic chamber method…and in general showed good agreement with flux measurements in the field (van Dijk et al., 2002; Rummel et al., 2002)."

Comment:
While the physical appearance of biological soil crusts is a useful classification tool, it does not provide any information on the actual nitrification processes that occur in or below the biocrust and may be responsible for controlling soil emissions of HONO and NO. Much of the molecular biology that is important for atmosphere-

land interactions is likely occurring just belowground (i.e., below the crust that is visible at the surface). It is also misleading to focus solely on the moss, lichen, actinobacteria, which are not the direct sources of these gases. Although biocrusts affect nutrient availability via N fixation, it is their possible associations with ammonia (and nitrite) oxidizing microbes (bacterial and archaea) that ultimately convert the fixed nitrogen to nitrite and nitrate. The current study does not consider the role of ammonia oxidizing microbes in association with biocrusts or the other surface types in the area. These microorganisms are not limited to living within or under biocrusts, but are present in most other soil types to differing degrees. It does make sense that such nitrifying organisms will thrive where their substrates are abundant. However, there are numerous other soil types where this may be the case. Further, there may be many other soil organisms that compete with nitrifiers for their substrates, that may reduce their abundance in soil that would seemingly favor nitrifier populations. The literature that does exist (e.g., Frontiers in Microbiology 2016, doi:10.3389/fmicb.2016.00505) on biocrust-nitrifier associations suggests that biocrusts do not necessarily host a greater abundance of ammonia oxidizing organisms compared to soil supporting trees, nitrogen fixing shrubs, etc. This is an important topic to address.

Response:
Thank you very much for that very good comment. The focus of the current study was to representatively quantify the HONO and NO emissions from the soil/biocrusts and to estimate their importance to the HONO budget by comparing these with the observed missing source. The underlying biological mechanisms were not focus of the current study and thus not discussed at greater detail.
It indeed is true that the dominating photoautotrophic compound doesn't tell us much about the microbial community below these, although, as suggested by the referee, these may have an effect on the belowground microbial community. A problem is, that different biocrust types could be distinguished in the field based on the dominating photoautotrophic compound, whereas microbial communities below the surface could not be determined by non-destructive methods. Within biocrusts, nitrification (and other nitrogen cycling processes) are expected to occur and the relevance of these processes is expected to be also substrate dependent (i.e. depending on the amount of ammonia present for nitrifiers to be used). We agree with the referee that these mechanisms are not restricted to biocrusts, but universally may also occur in non-crusted soils.
In the revised manuscript the following was added:
Chapter 1: "But much of the molecular biology/chemistry that is important for atmosphere-land interactions is likely occurring just below the crust (that is visible at the surface)."
Chapter 3.2: "The different biocrust types were distinguished in the field based on the dominating phototrophic compound but which provides no information about the microbial community below or about the magnitude of (de)nitrification processes. The microbial community couldn´t be determined by non-destructive methods."
Chapter 3.3: "Furthermore it was not possible to determine the microbial community below the biocrust or in bare soil. Although biocrusts increase nutrient availability via N fixation, it is their possible associations with ammonia oxidizing microbes (bacterial and archaea) that finally convert the fixed nitrogen to nitrite and nitrate. Nitrification and other nitrogen cycling processes are not restricted to biocrusts, but can also occur in non-crusted soils. The relevance of these processes is expected to depend on substrate richness (i.e. amount of ammonium available for nitrifiers)."

Comment:
Related to this, Figure 3 of the current manuscript demonstrates that there are other soil types throughout the landscape characterized by stones, litter, and vegetation cover that do not have associated flux values and were not included in the final conclusion regarding relative importance of biocrusts in HONO and NO emissions. The model only considered the approximately 45% of the surface types whose fluxes were characterized. It is possible that fluxes in the other soil types had as high or higher fluxes? If so, would this not make the estimate of contributions of soil emissions to overall atmospheric composition higher and possibly overshoot the Cyprus HONO budget determined in the field campaign? Indeed, Figure 8 is somewhat misleading since it must be noted that F only refers to the total HONO and NO flux associated with the 45% of surface types that were actual studied. It is very possible that the pie charts would look very different if other surfaces types were considered. So there is a large uncertainty here.*

Response:
To the best of our knowledge, there are no HONO and NO emissions from vascular vegetation, litter and stones. We also thoroughly searched the literature and did not find any publications showing emissions from these surface covers. This was also stated in the original manuscript "To the best of our knowledge, no data on reactive nitrogen emissions from vascular vegetation and plant litter have been published yet." (see original manuscript page 6, lines 14-15). Thus, we are very confident that F* (accounting for 45% of the total surface) represents the effective total emission from ground surface.

Comment:
In my opinion, a satisfying or conclusive connection between the soil emissions of NO and HONO and biocrusts has not been made. The most one can conclude from this study is that volatilization from soil bound nitrite could contribute to the NO and HONO measured in the air above the soil. Indeed, it may have been useful for the authors to include a better discussion of why they can rule out long range transport and atmospheric deposition of nitrate and NOx over time as the source of HONO and NO precursors to this soil. Even though this particular area of Cyprus may have a low population, is possible for it to accumulate anthropogenic inputs from population centers surrounding the Mediterranean basin over time? One is left wondering whether the results support the paper's title and the conclusions it suggests.

Response:
During the CYPHEX campaign (Meusel et al. 2016) very low NOx levels were detected (< 1ppb). Therefore deposition of NOx to the ground which could be converted into NO_2^-/NO_3^- and HONO was excluded as a relevant source. Also nitrate and ammonium concentrations in aerosol particles, ranging from 0.05-0.35 µg m^{-3} and 0.1-4 µg m^{-3}, respectively, (measured during CYPHEX) were too low to significantly account for HONO formation. It is not expected that the concentrations in this region are usually higher than found during CYPHEX. So deposition of reactive nitrogen species to the ground is low, and biologic processes (nitrification, denitrification) are the only reasonable explanations for HONO and NO emissions. The emissions were shown to be clearly linked to nutrient and particularly nitrite content Fig. 6), which in the current study seem to be driving HONO and NO emissions of crusted and non-crusted soils.
Please also check our response of referee #3 on [HONO]* calculations to answer the issue about simple volatilization from soil.
In the revised manuscript a short discussion was added:
"Nevertheless, a dominant contribution from microbial activity to the nutrient content is anticipated. Long range transport and atmospheric deposition of NOx and nitrate/nitrite/ammonium can be excluded to be a dominant source of HONO and NO precursors in local soil, as the observed concentrations in Cyprus ambient air were very low (Meusel et al., 2016; Kleanthous et al., 2014)."

Comment:
Lastly, Figure 2 presents a month of meteorological data (air and surface T, air and surface %RH, and precipitation) at the site for the month before samples were taken. The data features prominantly as Figure 2, yet is not used. So, it is unclear why an entire figure was devoted to this data when averages for these values during the time of sampling could have been provided in the text.

Response:
Agree, we now moved this figure to the supplement. Instead, we show a diel pattern of the mean surface temperature and RH with an estimated diel pattern for soil water content and simulated emissions (Fig. R2 or 8 in the revised manuscript; as suggested from referee #1).

Comment:
In conclusion, I feel that the strengths of this manuscript are that it is mostly well written and provides supporting evidence for the fact that soil emissions could have impacted the NOx and HONO budget during the CYPHEX 2014 field campaign. Weaknesses include: (i) there is minimal evidence from this study to support that

the emissions are biological in nature (outside of the fact that the flux vs. soil moisture plot matches those of studies on pure cultures of ammonia oxidizing bacteria, Oswald et al.) and (ii) there is less evidence that the actual biocrusts are the dominant HONO and NO sources in this area since we have no data on emissions from 55% of the other surface types present in the study area. Care must be taken here to not draw too much information from these results. The approach described in this paper is not unique; its novelty is related to providing data on soil HONO and NO emissions from understudied region of the globe. Due to its limited scope, this study would have been better suited as supporting data to include in the field campaign paper by Meusel et al. 2016. It may be possible for this study to stand on its own if the above concerns are appropriately addressed in a revised manuscript.

Response:

The aim of this study was not to prove the biological role or to characterize the biological mechanisms of HONO and NO emissions, but to show that soil and biocrust-covered soil in a remote (low pollution) area are an important HONO source (not differentiating between biological or solely physical exchange processes). The residual 55% of the surface coverage which was not studied in detail are very unlikely to emit significant amounts of HONO or NO, and no single study has indicated such an emission, so that the calculated F* is considered to be representative for the whole (local) surface.

Reference

Kleanthous, S., Vrekoussis, M., Mihalopoulos, N., Kalabokas, P., and Lelieveld, J.: On the temporal and spatial variation of ozone in Cyprus, Science of The Total Environment, 476–477, 677-687, http://dx.doi.org/10.1016/j.scitotenv.2013.12.101, 2014.

Meusel, H., Kuhn, U., Reiffs, A., Mallik, C., Harder, H., Martinez, M., Schuladen, J., Bohn, B., Parchatka, U., Crowley, J. N., Fischer, H., Tomsche, L., Novelli, A., Hoffmann, T., Janssen, R. H. H., Hartogensis, O., Pikridas, M., Vrekoussis, M., Bourtsoukidis, E., Weber, B., Lelieveld, J., Williams, J., Pöschl, U., Cheng, Y., and Su, H.: Daytime formation of nitrous acid at a coastal remote site in Cyprus indicating a common ground source of atmospheric HONO and NO, Atmos. Chem. Phys., 16, 14475-14493, 10.5194/acp-16-14475-2016, 2016.

Rummel, U., Ammann, C., Gut, A., Meixner, F. X., and Andreae, M. O.: Eddy covariance measurements of nitric oxide flux within an Amazonian rain forest, Journal of Geophysical Research: Atmospheres, 107, LBA 17-11-LBA 17-19, 10.1029/2001JD000520, 2002.

van Dijk, S. M., Gut, A., Kirkman, G. A., Gomes, B. M., Meixner, F. X., and Andreae, M. O.: Biogenic NO emissions from forest and pasture soils: Relating laboratory studies to field measurements, Journal of Geophysical Research: Atmospheres, 107, LBA 25-21-LBA 25-11, 10.1029/2001JD000358, 2002.

Weber, B., Wu, D., Tamm, A., Ruckteschler, N., Rodriguez-Caballero, E., Steinkamp, J., Meusel, H., Elbert, W., Behrendt, T., Soergel, M., Cheng, Y., Crutzen, P. J., Su, H., and Poeschi, U.: Biological soil crusts accelerate the nitrogen cycle through large NO and HONO emissions in drylands, Proceedings of the National Academy of Sciences of the United States of America, 112, 15384-15389, 10.1073/pnas.1515818112, 2015.