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Interactive comment on "Diversity and seasonal dynamics of airborne *Archaea*" *by* J. Fröhlich-Nowoisky et al.

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We thank the three anonymous referees for their constructive comments and suggestions, which are highly appreciated and have been taken into account in the revised manuscript. Detailed responses are given below. We will first focus on comments and suggestions within the general comment section and then on specific comments from the individual referees.

General comments

Anonymous Referee #2: Sanger Sequencing is no longer the preferred method of microbial ecological studies Anonymous Referee #3: I would echo the comments from Reviewer #2 regarding the use of Sanger sequencing

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Interactive Discussion

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Response: For an detailed answer concerning the choice of the sequence method we refer to the interactive comment on “Diversity and seasonal dynamics of airborne Archaea” by Ralf Conrad, in which he addressed in detail the reasons why Sanger Sequencing was chosen and why it also remains a sensible approach to our questions.

Anonymous Referee #2: Many samples of this study yielded very few clones and sequences (fewer than 1/ sample?, Table 1) perhaps due to inefficient cloning reactions or other laboratory issues, and it hardly seems fair to characterize any aspects of community composition based on such limited data Anonymous Referee #3: I would also echo the comments of Reviewer #2 regarding the issue around measurements with low sampling depth

Response: The authors agree with the referees comment that it is not possible to draw solid conclusions about the diversity of a population based on a few sequences only. However, the measured species richness S provided in Table 1 was mainly calculated for completeness and as a comparison for the two sample sets where more sequence data was gained. We did not discuss nor even mention the other locations concerning their diversity within the text and only use the few sequences to support the results we found in Germany and Cape Verde. However, to make it clearer that the measured species richness is not to be interpreted strongly we changed the Table head of Table 1 into: “. . ...obtained number of DNA sequences as well as the statistical parameters: species richness (S measured, S* estimated), Shannon index (H’), Shannon Evenness (E), and Simpson’s index (D), not available (n.a.), Table S3). The measured species richness S in North America, China, and UK needs to be interpreted with caution as only few sequences were available.”

Anonymous Referee #3: The sampling method should be mentioned much earlier in the text

Response: As also required by Anonymous Referee #2 the Sanger Sequencing method is now mentioned already in the abstract.

Anonymous Referee #3: It seems trivial to attempt to characterize the archaeal communities present in these samples base on so few sequences/ sample. I think the manuscript would be improved if these samples were omitted entirely as they do not add to the overall conclusions of the paper

Response: Several scientists, as pointed out in the manuscript, have difficulties in amplifying airborne Archaea. We thus believe, that even the few sequences gained from different sampling sites should be provided to the scientific community, as further studies can built upon these first preliminary results. Besides, the few sequences of the additional sampling site support the other finding of the manuscript as well as what currently could be gained from literature research. We do not over interpret the finding and stay very cautious every time we mention the finding, so that every reader can draw her/his own conclusion. We thus would like to retain the samples with few sequences in the manuscript. However, we followed the suggestion from Anonymous Referee #2 in moving the sampling strategy of these sites into the supplemental material as by this, the focus of the main paper is even more centered on Germany and Cape Verde.

Anonymous Referee #3: In addition it was not clear to me whey the authors chose to amplify and analyze amoA gene sequences. In the introduction (ln 57-58) the authors state that the atmosphere is likely not habitat of Archaea, so it is unclear to me why they chose to focus on metabolic groups of Archaea, specifically the ammonia oxidizers. Does information about metabolic groups explain the influence of possible source environmental structuring Archaeal communities?

Response: When analyzing the composition of airborne Archaea we detected primarily Thaumarchaeota. To strengthen this characterization based only on the 16S rRNA gene, we in addition studied the amoA gene which confirmed the taxonomic identification and thus presence of Thaumarchaeota in air. The answer to the referees comment is also addressed in the interactive comment by Ralf Conrad stating: "The relatively large frequency of archaeal sequences belonging to the thaumarchaeotal group is the reason why the amoA gene (coding for a subunit of the ammonia monooxygenase, the

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key enzyme of ammonia oxidizing nitrifiers), which characteristically occurs in Thaumarchaeota, was also sequenced. Targeting this gene provides additional phylogenetic information for this not well characterized archaeal group."

Specific comments

By Anonymous Referee #1

Anonymous Referee #1: I haven't found anywhere the detection limits of the qPCR assay for Archaea and Bacteria in the 16S gene.

Response: The general detection limit is 10 copies of DNA. These can be detected convincingly. We thus changed the sentence in the supplementary material discussing the detection limit into: "In contrast, Archaea 16S rRNA gene copies were often below the detection limit (< 10 copies), and varied approximately between 1 and 10 copies per m3 air."

Anonymous Referee #1: Also, please include Rˆ2 and efficiency in those qPCR reactions.

Response: The efficiency and correlation coefficient was and is given at the end of the second paragraph of the "Quantitative analysis of airborne Archaea" section in the supplementary material. If the Anonymous Referee would like them to be included in the main text, we can do this, but as all other results concerning the qPCR are discussed in the supplemental material, we would leave rather them in the supplemental material.

Anonymous Referee #1: Typing error: database (not "data base")

Response: The typing error was corrected throughout the revised manuscript

Anonymous Referee #1: line 26, page 6964: Thaumarchaeota

Reponse: The typing error has been corrected in the revised manuscript.

By Anonymous Referee #2

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Anonymous Referee #2: The first sentence of the abstract should clarify that these abundances are observed outside extreme environments.

Response: We changed the first sentence of the abstract as follows: "Archaea are widespread and abundant in many terrestrial and aquatic environments, thus outside extreme environments, accounting for up to ~10% of the prokaryotes."

Anonymous Referee #2: The use of Sanger sequencing is a surprise encountered well in the manuscript. The sequencing approach should be made clear upfront, preferable in the abstract. The number of sequences per site or sample for the main sites should also be mentioned (Cape Verde, Mainz)

Response: We followed the Anonymous Referees advice and changed the third sentence in the abstract as follows: "By DNA analysis and Sanger Sequencing targeting the 16S rRNA and amoA genes in samples of air particulate matter collected over one year at a continental sampling site in Germany, we obtained first insights into the seasonal dynamics of airborne Archaea". We also added the number of sequences for Germany and Cape Verde in the abstract.

Anonymous Referee #2: Beginning in the abstract, all mention of methanogens and ammonia oxidizers should be changed to "predicted methanogens" or "predicted ammonia oxidizers" or similar, as functional information cannot be determined from your approach, even from amoA genes, as you correctly indicate in in 4 towards the end of introduction.

Response: Following the referees advice, throughout the manuscript the description of methanogens and ammonia oxidizers has been formulated as suggested.

Anonymous Referee #2: a) Introduction Line 15 and other places that consider previous studies on airborne archaea: please also cite "Bowers et al 2013, Env Sci Tech. Though archaea were not the focus of that work, they were picked up by universal primers and are shown in Fig 1A and mentioned briefly towards the beginning of the

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Results section.

Response: The publication of Bowers et al 2013 was inserted.

Anonymous Referee #2: b) This study should also be added to Table 2, though much of the data in the table were not reported, so that is up to you.

Response: Within Table 2, we included a "*" at the HiSeq Illumina technique and explain the "*" in the caption of Table 2, by mentioning the Archaea findings in Bowers et al 2013 paper, but also stating that not enough information is included in the paper itself to present it in the table properly. Within the table caption it now says: ". . ..another study by Bowers et al (2013) analyzed bacteria and fungi in air collected at the Colorado Front Range over a 14 month period with the Illumina HiSeq technique. Within this study Archaea were amplified as well, however, no detailed information is provided."

Anonymous Referee #2: c) The observed low relative abundance of archaea in that study is in agreement with your qPCR data and should be cited in relation to that as well (section 3.1, ln 10-15

Response: We included the Bowers et al 2013 reference in the supplemental material in the second before last paragraph of the discussion of "quantitative analysis of airborne Archaea". We also changed the main text and added at the end of the first paragraph in section 3.1. the sentence: "The observation that Archaea are more difficult to amplify than other airborne microbes has been stated in other publications as well (Bowers et al., 2009, 2013; Fierer et al., 2008; Woo et al., 2013, Woo, 2012).

Anonymous Referee #2: I suggest moving all of the text describing sampling methods for the sites that yielded few sequences to the supplementary material. You (rightly) do not focus on the other sites in the main text, and the methodological details are distracting. Table 1 can include all sites.

Response: At the end of the first paragraph of section 2.1 we included the following sentence "With the main analyses focusing on the samples from Germany and Cape

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Verde only the detailed sampling strategies for these sites are presented here, while the information for the other sites is available in the supplementary material." The information on the other sites (North America, China, and United Kingdom are shifted to the supplementary material under the caption. "Aerosol sampling at additional sites".

Anonymous Referee #2: The sampling methods for each site are quite different. Table 1 helps the reader to understand some of these differences but additional information should be added to Table 1, e.g. the filter type used, the sampling duration (on average or range), average flow rates.

Response: Within Table 1 the sampling time, filter type, and the average flow rate were added for all sites as requested by the reviewer

Anonymous Referee #2: Along these lines, section 3.4, lines 18-19 is simply not true and should be cut.

Response: The sentence was cut as suggested by the Anonymous Referee.

Anonymous Referee #2: line 20: What does shipped at reduced temperatures mean?

Response: This sentence is now found in the supplementary material under "United Kingdom". It was changed into: "...loaded filters were transported frozen and stored in a freezer at -20°C until DNA extraction."

Anonymous Referee #2: Lin 8: what buffer?

Response: The sentence was changed into: ...after lysis, followed by addition of 900 μl buffer provided by the extraction kit....

Anonymous Referee #2: Line 15: A maximum of 4 PCR reactions, but a minimum of how many? What is the justification of different numbers of PCR reactions per samples

Response: To clarify the number of PCRs we added the following sentences: As it was difficult to amplify airborne Archaea (see also supplementary material) we used to increase the amplification success 3 primer pairs as well as nested primers. Thus,

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with the DNA from each of the filters up to four polymerase chain reactions (PCR) were performed using normal and nested primer pairs targeting the 16S rRNA gene. The number of PCRs was dependent on the successful amplification of the previous PCRs. To detect even minimal amounts of archaeal DNA, an additional PCR and eventually nested PCRs were only performed in case the first PCR failed.

Anonymous Referee #2: Second page line 17: What does "each PCR product "mean? Each sample? Each pooled quadruplicate qPCR reaction? Each site?

Response: To clarify this question we changed the first sentence of the last paragraph of section 2.2 into: "Amplification products of each filter sample selected for sequencing were cloned using the TOPO TA Cloning Kit (Invitrogen) following the supplier's instructions"

Anonymous Referee #2: Also, please, be clear about the efficiency of the cloning reactions indicating somewhere (Table 1?) how many colonies were generated for each sample and site and how many were picked

Response: We integrated the following sentence in the last paragraph of section 2.2. "The cloning efficiency was between 5 and 200 colonies per cloned PCR product."

Anonymous Referee #2: Line 13-15. I am not convinced that you have the statistical power to make accurate richness measurements or predictions, based on such a small number of sequences. That caveat should be mentioned (here or in the results/ discussion) and you should clarify that you only made these predictions for sites with the most sequences (Mainz and Cape Verde, best I can tell).

Response: We added the following paragraph at the beginning of section 2.5.: "Although the number of the here analyzed Sanger sequences is compared to modern NGS techniques low, we performed statistical analyses to get a first indication of species richness and possible correlations with meteorological conditions. However, species richness measurements were only performed for the sites with most se-

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quences (Germany and Cape Verde). Correlation analyses with meteorological conditions were only performed for Germany, where most sequences were analyzed. “

Anonymous Referee #2: p. 6959 near line 5: please also cite Brochier-Armanet, 2008, Nat Rev Microbio here.

Response: The reference was included.

Anonymous Referee #2: p. 6960 line 25-28: this is highly speculative, considering the data set. I recommend cutting this.

Response: The sentence was deleted as suggested by the reviewer.

Anonymous Referee #2: Line 19 “occurred” should be changed to “were detected”

Response: Changes were made according to the referee’s suggestion.

Anonymous Referee #2: Line 15: consider adding a citation to Bowers et al 2011, ISMEJ

Response: The citation of Bowers et al 2011 was included and the according sentence changed as follows: “Initial analyses suggest that the composition of airborne microorganisms shows biogeographic patterns especially between continental, coastal, and marine sites but also across different land-use types , though only a few studies have considered, or attempted to study this phenomenon (Bowers et al., 2011; Fröhlich-Nowoisky et al., 2012; Womack et al., 2010)”

Anonymous Referee #2: p. 6965, lines 13-14: unless you had a direct correspondence with Brodie et al or reanalyzed their data (if so please state) you cannot confirm that the Crenarchaeota observed in that study have been reclassified as Thaumarchaeota. That may be a reasonable assumption but this sentence should be rephrased

Response: To make clear that we think this assumption to be reasonable but have not actually analyzed the data from Brodie et al, we changed the sentence into: “Using microarray techniques targeting 16S rRNA genes, Brodie et al (2007) counted, in

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two cities in Texas, 30% Crenarchaeota (under the assumption that these would in a reanalysis be assigned to Thaumarchaeota) and 70% Euryarchaeota"

Anonymous Referee #3

Anonymous Referee #3: Line 66 Other studies that detected Archaea in air samples include Yooseph et al 2013 and Robertson et al 2013

Response: Yooseph et al 2013 and Robertson et al 2013 were added.

Anonymous Referee #3: Line 194-201 Why were two set of 16S primers used? This is addressed in the supplement but I suggest briefly mentioning it in the main text

Response: In the main text we added in the paragraph where we start describing the PCRs the following sentence: "As it was difficult to amplify airborne Archaea (see also supplementary material) we used to increase the amplification success 3 primer pairs as well as nested primers. Thus, with the DNA extract. . .."

Anonymous Referee #3: Line 322-325 I recommend removing this sentence unless there are other studies to support these claims

Response: The original sentence was: "However, the fact that Archaea were more numerous on coarse filters may indicate that airborne Archaea are mostly not isolated cells, with typical diameters between 1 and 2 μm, but rather cells that cluster together or aggregate with other particles like soil dust and thus deposit faster than Bacteria (for details see supplementary material)." This sentence was now replaced by: "However, the fact that Archaea were more numerous in coarse particle filters may indicate that Archaea are often attached to soil dust particles as it also has been shown for Bacteria (Jones and Harrison, 2004)."

Anonymous Referee #3: Line 450-451 also see Bowers et all 2011, also previous work by this group Despres et al 2007, there are also several studies using culture-based approaches including Shaffer& Liughthard 1997 or Bovallius et al 1978

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Response: We integrated the references of Despres et al 2007; Shaffer et al 1997 and Bovallius et al 1978 and changed the precious sentences as follows: "….though only a few studies have considered, or attempted to study this phenomenon with molecular or cultural methods (e.g., Bovallius et al., 1978; Bowers et al., 2011; Després et al., 2007; Fröhlich-Nowoisky et al., 2012; Shaffer and Lighthart, 1997; Womack et al., 2010)"

Anonymous Referee #3: Line 454-456: sentence wording is unclear.

Response: To make the sentence clearer we changed it as follows: "Although in a comparison between sites the local geographic and meteorological conditions need to be taken into account, the height in which the samples were taken for this study is negligible as the continental boundary layer air up to 1000 m is fairly well mixed and all sorts of atmospheric measurements can be compared."

Anonymous Referee #3: Line 490- capital "C"

Response: The change has been made as suggested by the referee.

Anonymous Referee #3: Table 2: San Antonio, Texas & Mt Bachelor, Oregon

Response: According to the referee's suggestion the spelling of "San Antonio" in Table 2 has been corrected and "Oregon" added.

Anonymous Referee #3: It is my understanding that Archaea/Archaeal should not be italicized as italicized fonts are typically reserved for genus (species) names.

Response: "Archaea" and "archaeal" have been adjusted to the referee's recommendations and are now written "regular" throughout the text and also the supplemental material. Similar "Bacteria, bacterial, Fungi, and fungal" have been changed from italicized writing to regular style.

Interactive comment on Biogeosciences Discuss., 11, 6945, 2014.

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