

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

**Anonymous Referee #2**

Received and published: 15 June 2014

General comments:

Fröhlich-Nowoisky and colleagues present research on an important and understudied component of atmospheric bioaerosols, the archaea. They are to be commended for the clarity of their writing. Overall, this is an important contribution, but I found two aspects of the work disappointing. First, Sanger sequencing is no longer the preferred method for microbial ecological studies. Most studies now use next-gen sequencing approaches, preferably Illumina, which allow for a much deeper interrogation of microbial community structure. Second, many of the samples in this study yielded very few clones and sequences (fewer than one per sample in some cases, according to 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

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such limited data. Most Illumina datasets would be rarefied to 1000 or more sequences per sample.

Specific comments:

The first sentence of the abstract should clarify that these abundances are observed outside extreme environments.

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

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 ln 4 towards the end of the Introduction.

Introduction ln 15 and other places that consider previous studies on airborne archaea: please also cite Bowers et al. 2013, Environmental Science & Technology. Though archaea were not the focus of that work, they were picked up by the universal primers and are shown in Fig. 1A and mentioned briefly towards the beginning of the Results section. This study could also be added to Table 2, though much of the data in the table were not reported, so that is up to you. 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).

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

The sampling methods for each site are quite different. Table 1 helps the reader to

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understand some these differences, but additional information should be added to this table, such as the type of filter used, sampling duration (on average and/or the range), and average flow rate. Along these lines, section 3.4, In 18-19 is simply not true and should be cut.

2.1.5, In 20: What does shipped at reduced temperatures mean?

2.2, In 8: What buffer?

2.2, In 15: A maximum of 4 PCR reactions, but a minimum of how many? What is the justification for different numbers of PCR reactions per sample?

2.2, second page, In 17: What does each PCR product mean? Each sample? Each pooled quadruplicate qPCR reaction? Each site? Also, please be clear about the efficiency of the cloning reactions, indicating somewhere (Table 1?) how many colonies were generated for each sample and/or site and how many were picked.

2.5 In 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 and/or in the Results/Discussion), and you should clarify that you only made these predictions for site(s) with the most sequences (Mainz and Cape Verde, best I can tell).

3.1, p. 6959, near In 5: please also cite Brochier-Armanet, 2008, Nat Rev Microbio here.

p. 6960, In 25-28: this is highly speculative, considering the dataset. I recommend cutting this.

3.3, In 19: "occurred" should be changed to "were detected"

3.4, In 15, consider adding a citation to Bowers et al., 2011, ISMEJ

p. 6965, In 13-14: unless you had a direct correspondence with Brodie et al. and/or reanalyzed their data (if so, please state), you cannot confirm that the Crenarchaeota

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observed in that study have been reclassified as Thaumarchaeota. That may be a reasonable assumption, but this sentence should be rephrased.

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

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