Responses to the reviewer 1 comments

Renard et al. present interesting information about the composition of free amino acids (AA) in cloud water from a French mountain site. The LC-MS analytical work is sophisticated and sensitive. Care is taken to avoid important artifacts associated with matrix effects commonly found in LC-MS analysis of complex environmental samples. The results are novel, and the authors do a good job exploring several hypotheses related to factors influencing the abundance and relative composition of the observed AA. I do have several suggestions to improve the manuscript prior to publication.

We would like to thank the reviewer for this positive comment and for the suggestions. This will improve the quality of the manuscript. We answer to your comments below.

1. Please provide more detail about experimental methods:

- Add a short description of the cloud water collectors

We modified the text as follows:

Line 115:

"The sampling is performed using aluminum cloud water collectors under non-precipitating and non-freezing conditions as described in Deguillaume et al. (2014). Cloud droplets are collected by impaction onto a rectangular plate which then flows directly into a sterilized bottle going through a funnel. The impactor has an estimated cut-off diameter of 7 µm. Before cloud collection, cloud impactors are cleaned using milliQ water and sterilized by autoclaving. Immediately after sampling, a fraction of the aqueous volume is filtered using a 0.2 µm nylon filter (Fisherbrand™) to eliminate microorganisms. The samples are then stored in the dark and frozen at -20 °C (ATP, ion chromatography, total organic carbon, and amino acids). For cell counts, samples are stored at 4 °C after adding a fixative. The analyzes are performed shortly thereafter."

- Provide more detail about the instruments and materials used, such as the manufacturer of the nylon filter and the model of the Shimadzu TOC analyzer

We modified the manuscript as follows:

Line 119:

"0.2 µm nylon filter (Fisherbrand™)"

Line 128:

"TOC analyses are performed with a TOC analyzer (Shimadzu, TOC-5050A)."

- Line 118: be more specific about storage temperature and storage times prior to analysis for particular analytes, especially the AAs.

For the measurements of ATP and chemical compounds (ionic chromatography, total organic carbon, and amino acids), samples were kept in the dark at -20 °C before their analyses in the following months. For the counting of bacterial cells, samples were stored in the dark at 4 °C after the addition of a fixative agent (glutaraldehyde, 0.5 % final) before their analyses in the following weeks.

We modified as follows:

Line 120:

"The samples are then stored in the dark and frozen at -20 °C (ATP, ion chromatography, total organic carbon, and amino acids). For cell counts, samples are stored at 4 °C after adding a fixative. The analyzes are performed shortly thereafter."

- Please add a reference for the Gerber Scientific PVM-100.

The reference *Gerber* (1991) has been added in the manuscript.

Line 133:

"Gerber, H.: Direct measurement of suspended particulate volume concentration and far-infrared extinction coefficient with a laser-diffraction instrument, Appl. Opt., 30, 4824-4831, 1991."

2. The authors compare AA compositions of cloud water samples collected over 6 years. Are they confident that differences in AA abundance and relative composition are not affected by differing storage times/conditions?

Cloud samples are filtered to remove microorganisms before dark storage at -20 °C. Under these conditions, biotic or abiotic transformations should be negligible. In addition, as mentioned in line 120, the time between cloud collection and their analyzes was constant throughout the sampling period. With the exception of the 22-Mar-14 sample, AA measurements were conducted a few months after sampling. We kept the latter because it did not show significantly lower AA concentrations (Kruskal-Wallis test, p-values <0.05) than the samples from 2018, 2019 and 2020.

3. Line 223 and abstract: Standard addition prevents introduction of analytical biases resulting from matrix effects. It does not actually prevent the matrix effects.

You are right, the term "avoid" is probably excessive. Hewavitharana et al. (2018) used the term "overcome".

We modified as follows:

Line 14:

"This quantification has been performed without concentration neither derivatization, using LC-MS and the standard addition method to correct for matrix effects."

Line 101:

"In addition, to overcome matrix effect, we propose to quantify the AAs by the standard addition method (Hewavitharana et al., 2018)."

Line 230:

"The standard addition method also restrains matrix effects which are very commonly encountered with environmental matrices (Hewavitharana et al., 2018)."

4. The authors need to better distinguish the two sections of the manuscripts discussing STD. On p. 7, it would help the reader if they clearly indicated that they are speaking about the precision of the measurements of AA while they later discuss the variability of concentrations across different cloud samples. In both places the authors tend to rely on the jargon of discussing STD. More nuance in the descriptions would help.

To distinguish the standard deviation describing the precision of the measurements (STD_M), from the standard deviation describing the variability of concentrations across different cloud samples (STD), we replaced STD by STD_M in the section 3.1 ("Evaluation of LC-MS technique for a direct measurement of AAs in cloud"), and modified the text as follows:

Line 232:

"Concentrations values obtained for all AAs and cloud samples – as well as the standard deviation of the measurements (STD_M) (i.e., the precision of the measurements of AA concentrations) – are reported in Table S3 and detailed in Figure S3."

5. The authors are correct that not many publications have reported concentrations of speciated AA in cloud, fog, or rain. They did, however, overlook an early, seminal paper by Mopper and Zika (1987) Nature 325, 246-249. They should review this early paper, add it to their comparison tables, and include its findings in their discussions of comparisons to their current work.

You are right. Mopper and Zika (1987) reported AA concentrations in their paper. We had taken their results into account, and we had mentioned them in the introduction (lines 70 and 95), in the section 3.4 ("Comparison with previous studies on clouds, fogs, and rain") (Table 2), in the section 4.2 ("Potential influence of the air mass origin on the AA concentrations and their relative distribution") (line 465) and in the SI (Table S4).

6. Please replace "hydropathy" with hygroscopicity throughout. Replace "multiphasic" with multiphase.

This has been replaced in all the manuscript.

7. The authors switch between referring to the Cape Verde Islands and the Cabo Verde Islands. Please switch all to Cape Verde Islands.

We have replaced "Cabo Verde" by "Cape Verde".

8. Please change terminology so that you refer to trajectories within (not below) the boundary layer.

We used either "below the ABLH" (consistent with the model), or "inside the ABL".

9. Liquid chromatography is coupled to mass spectrometry, not "hyphened to" it.

We modified the text as indicated.

10. The manuscript contains numerous errors in grammar and syntax and several awkwardly phrased sentences. There are also unusual choices to capitalize certain words (e.g., Sea, Free, Continental, all of which should not be capitalized), some misspellings (e.g., De Hann), many cases of singular-plural disagreements between nouns

and verbs, and numerous poor choices of prepositions. With a few exceptions, the authors' meaning is clear, but the text would greatly benefit from English language editing.

We will contact after the reviewing process the ACP editing service to improve the quality of the manuscript. Therefore, the errors in grammar and syntax will be corrected.

References

Deguillaume, L., Charbouillot, T., Joly, M., Vaïtilingom, M., Parazols, M., Marinoni, A., Amato, P., Delort, A.-M., Vinatier, V., Flossmann, A., Chaumerliac, N., Pichon, J. M., Houdier, S., Laj, P., Sellegri, K., Colomb, A., Brigante, M., and Mailhot, G.: Classification of clouds sampled at the puy de Dôme (France) based on 10 yr of monitoring of their physicochemical properties, Atmos. Chem. Phys., 14, 1485–1506, https://doi.org/10.5194/acp-14-1485-2014, 2014.

Gerber, H.: Direct measurement of suspended particulate volume concentration and far-infrared extinction coefficient with a laser-diffraction instrument, Appl. Opt., AO, 30, 4824–4831, https://doi.org/10.1364/AO.30.004824, 1991.

Hewavitharana, A. K., Abu Kassim, N. S., and Shaw, P. N.: Standard addition with internal standardisation as an alternative to using stable isotope labelled internal standards to correct for matrix effects-Comparison and validation using liquid chromatography-tandem mass spectrometric assay of vitamin D, J Chromatogr A, 1553, 101–107, https://doi.org/10.1016/j.chroma.2018.04.026, 2018.

Mopper, K. and Zika, R. G.: Free amino acids in marine rains: evidence for oxidation and potential role in nitrogen cycling, 325, 246–249, https://doi.org/10.1038/325246a0, 1987.