Reply to Smith and Griffin: Methods, air flows, and conclusions are robust in the DeLeon-Rodriguez et al. study

Smith and Griffin (1) raise four concerns regarding our study of the microbiome of the upper troposphere (2). Sampling microbial cells in this environment remains technically challenging, but the concerns raised are not substantial for the reasons stated below. Our methods were adequate and our conclusions well supported by the available data.

In response to Smith and Griffin’s (1) first concern, we provided enough information for our air-sample collection system to be reproduced; the brand of the pump does not represent critical information and can be obtained from the authors upon request. The system has also been described, in detail, previously (3). Briefly, air was collected through a specialized inlet probe from the free airstream, well outside the aircraft surface boundary layer. The sample collection system was operated at or below ambient pressures and the entire system was leak-checked periodically by (i) closing off the inlet and vacuum flow and monitoring the rise in system pressure with time, and (ii) periodically introducing a particle filter into the inlet flow and monitoring particle counts in the downstream air with a condensation nuclei counter. Brand-new (sterilized) sampling lines were used in this campaign to minimize contamination issues and no microbial biofilm was visually detectable on tubing surfaces. If there were contamination of the line or a systematic sampling artifact (e.g., microbes attached on the walls of the sampling line or airplane inlet during take-off and landing) we would have detected the corresponding microbes, in high abundances, in all flights/samples, because most of our flights began from the same airport. No such patterns were observed. In addition, handling blank samples were obtained to establish background bioaerosol counts (2).

Concerning Smith and Griffin’s (1) second point, the difference in cell numbers may be simply attributable to the different techniques used in our respective studies; for example, filters and microscopy techniques used by Smith and Griffin can underestimate cell numbers, as these authors also stated in their report (4), or the different air masses sampled (e.g., we sampled air perturbed by hurricanes). Furthermore, other studies (5, 6) reported similar bacterial cell numbers at high altitudes to those reported in our article. Finally, the “276%” bacterial contribution to total particles quoted for one sample (1) is presumably because of the uncertainty/noise of the measurements of total particles or microscopy counts as we discussed, in detail, previously (2). Our results are semiquantitative and the major conclusion of our study—that bacterial cells are within the same order of magnitude as abiotic particles—remains well supported by several samples.

In terms of temperatures, Smith and Griffin’s (1) third concern, the temperature in the altitudes sampled, 8–10 km, is −35 °C to −45 °C and this represents common knowledge. To the best of our knowledge, whether or not microbes can be metabolically active under such atmospheric conditions or during updraft has yet to be tested.

Finally, the goal of our study was obviously not to evaluate annual or seasonal variability of microbial communities and their respective concentrations.

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