Monitoring changes in the composition of microbial communities by air-sampling and nanopore sequencing

The composition of airborne microbial populations changes continually. Air-sampling and sequencing provide a way to identify the species present, potentially allowing rapid action to be taken

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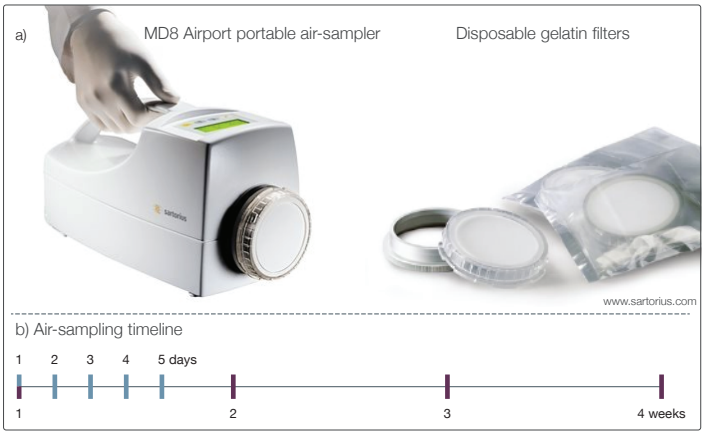

Fig. 1 Air sampling a) equipment b) sampling timeline

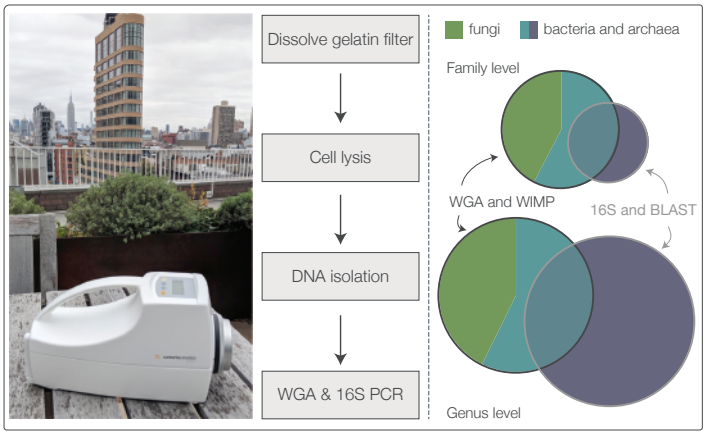

Fig. 2 Air sampling location, workflow and results from a single timepoint

Air-sampling and sequencing allow us to monitor environmental microbes in real time

Air-sampling (Fig. 1) followed by culturing has traditionally been used in the pharmaceutical industry as a way of assessing and monitoring the cleanliness of areas used in manufacturing processes. The same approach is also increasingly being used to evaluate air cleanliness in hospital operating theatres, sites where food is manufactured, and office ventilation space. However, many micro-organisms have specific nutritional or environmental growth requirements and cannot easily be cultured, meaning that a culture-based monitoring strategy provides an incomplete picture of the organisms present. In addition, culturing is a time-consuming process and does not allow rapid action to be taken.

Composition of microbial communities using 16S and whole-genome sequencing

Genomic analysis by nanopore sequencing provides a powerful, sensitive and rapid method to analyse changes in the composition of airborne microbial communities, without the need to culture. To monitor changes in the relative abundance of different airborne microorganisms over time, we sampled the air around the Oxford Nanopore office in Lower Manhattan over daily and weekly time intervals using a Sartorius MD8 air sampler equipped with gelatin filters. For each timepoint we sampled air at a rate of 50 dm^3 per minute for 20 min. Following sampling we dissolved the gelatin filter, recovered and lysed the cells that had become attached to it and extracted total genomic DNA (Fig. 2).

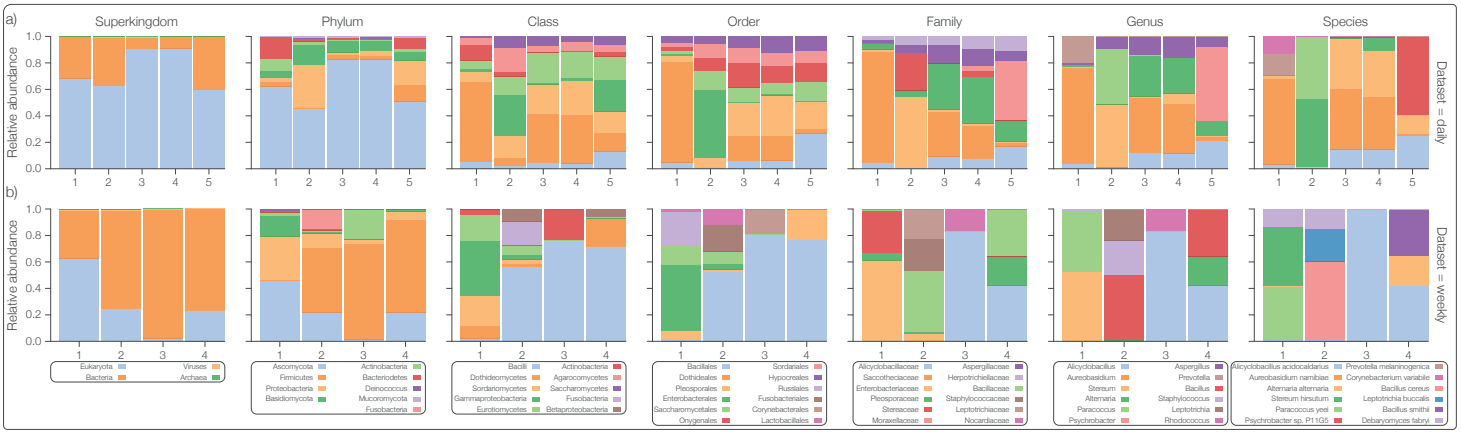

Fig. 3 Changes in the composition of the airborne microbial community near ONT's NY office, at different taxonomic levels a) sampled each day for 5 days b) sampled once a week for 4 weeks

Longitudinal analysis of airborne microbes by air-sampling, whole-genome amplification and nanopore sequencing reveals large fluctuations in community composition over time

We identified the organisms present in two ways: BLASTN analysis of 16S amplicons to identify bacteria and archaea, and WIMP analysis of whole-genome amplified libraries to identify bacteria, archaea, fungi and viruses. A large number of fungal and bacterial genera were found. The overlap between bacteria identified by the two approaches was good, but each approach also identified many unique families and genera, which is partly explained by the taxonomic coverage of the respective reference databases (Fig. 2). Interestingly, no plant species were detected in the data, a result which we confirmed by 18S qPCR. Given the likelihood of pollen grains being captured during air-sampling it is probable that the cell lysis conditions used here were insufficient to lyse plant cells. Following WIMP analysis, we took the csv file from the EPI2ME output to compare results from each timepoint at different taxonomic levels. The composition of sampled air changes markedly on daily and weekly timeframes, with weekly changes being more substantial (Fig. 3). The combination of air sampling and whole-genome sequencing could be used to monitor the presence of airborne bacteria, fungi and viruses which are potentially pathogenic to humans, whether these changes result from natural influences or human activities. Whole-genome sequencing provides a much richer dataset than 16S sequencing, giving the user information on gene families that cannot be inferred from a single-gene analysis, such as antimicrobial resistance. The method can also be used to detect airborne crop pathogens, which could enable better pesticide stewardship, allowing earlier and more targeted treatment of crops, and avoiding the application of pesticides to which there is resistance.