## Background

Establishing reliable methods for assessing the microbiome within the built environment is critical for understanding the impact of biological exposures on human health. High-throughput DNA sequencing of dust samples provides valuable insights into the microbiome present in human-occupied spaces. However, the effect that different sampling methods have on the microbial community recovered from dust samples is not well understood across sample types. Heating, ventilation, and air conditioning (HVAC) filters hold promise as long-term, spatially integrated, high volume samplers to characterize the airborne microbiome in homes and other climate-controlled spaces. In this study, the effect that dust recovery method (i.e., cut and elution, swabbing, or vacuuming) has on the microbial community structure, membership, and repeatability inferred by Illumina sequencing was evaluated.

## Results

The results indicate that vacuum samples captured **higher quantities of total**, **bacterial**, and **fungal DNA** than swab or cut samples. Repeated swab and vacuum samples collected from the same filter were less variable than cut samples with respect to both quantitative DNA recovery and bacterial community structure. Vacuum samples captured substantially greater bacterial diversity than the other methods, whereas fungal diversity was similar across all three methods. Vacuum and swab samples of HVAC filter dust were repeatable and generally superior to cut samples. Nevertheless, the contribution of environmental and human sources to the bacterial and fungal communities recovered via each sampling method was generally consistent across the methods investigated.

## Conclusions

Dust recovery methodologies have been shown to affect the recovery, repeatability, structure, and membership of microbial communities recovered from dust samples in the built environment. The results of this study are directly applicable to indoor microbiota studies utilizing the filter forensics approach. More broadly, this study provides a better understanding of the microbial community variability attributable to sampling

Support provided by:

methodology and helps inform interpretation of data collected from other types of dust samples collected from indoor environments.
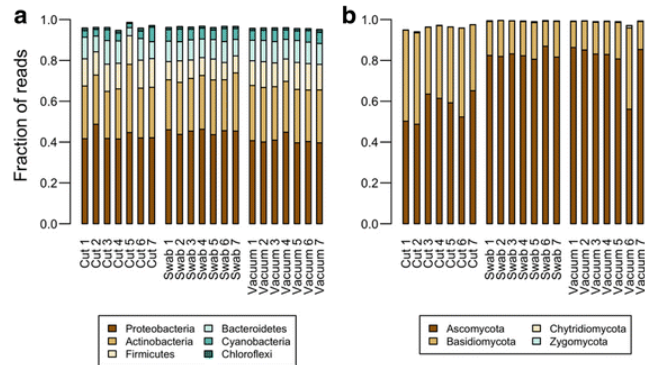


**Figure 1.** Relative abundance of top bacterial phyla (**a**) and fungal phyla (**b**) per sample. Selected taxa were top six groups with fully identifiable phylogeny and were ranked by median abundance.
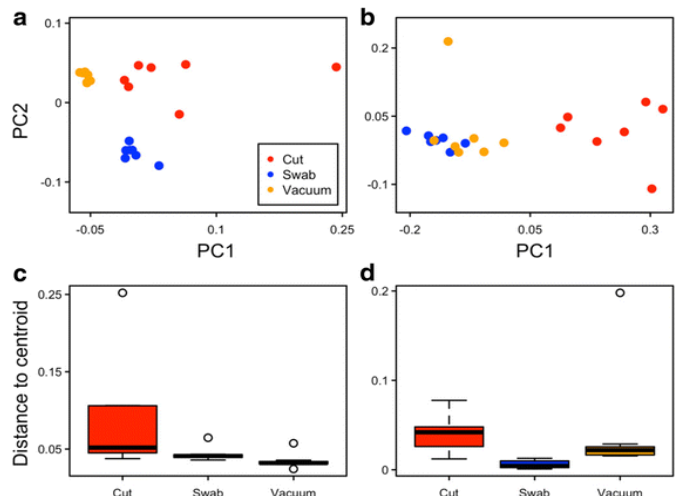


**Figure 5.** Community repeatability by sample type. PCoA plots for (**a**) bacteria (variance explained PC1 = 44% and PC2 = 22%) and (**b**) fungi (variance explained PC1 = 59% and PC2 = 9%) are based on weighted UniFrac and Morisita-Horn distances, respectively. Boxplots for (**c**) bacteria and (**d**) fungi represent distances to group centroids produced by the betadisper function in Vegan, which runs an analysis of multivariate homogeneity of dispersions. PCoA = principal component analysis.