Anthropogenic Impact on environmental Microbiome using Nanopore Metagenomics

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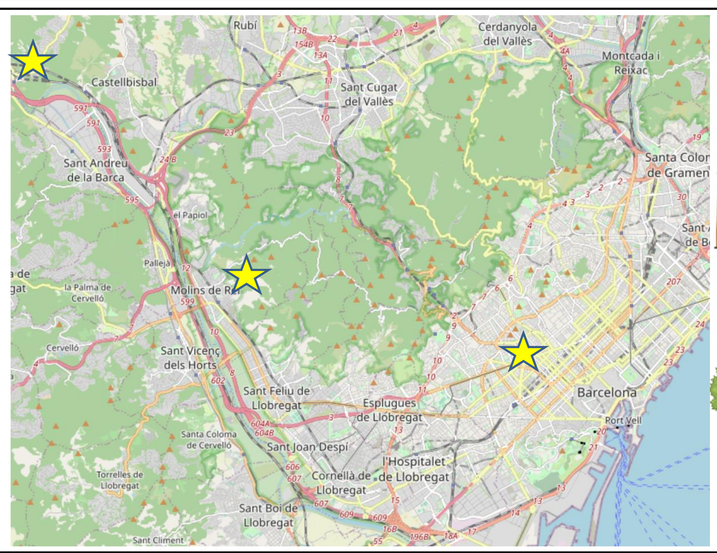
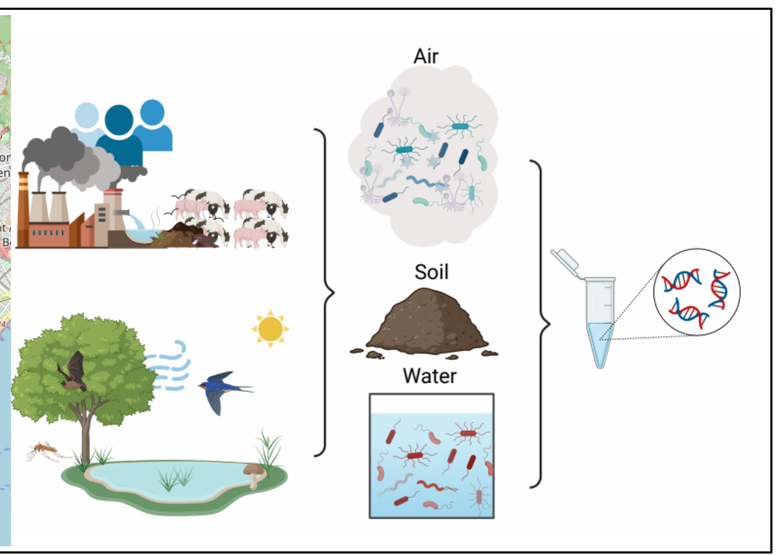
Background

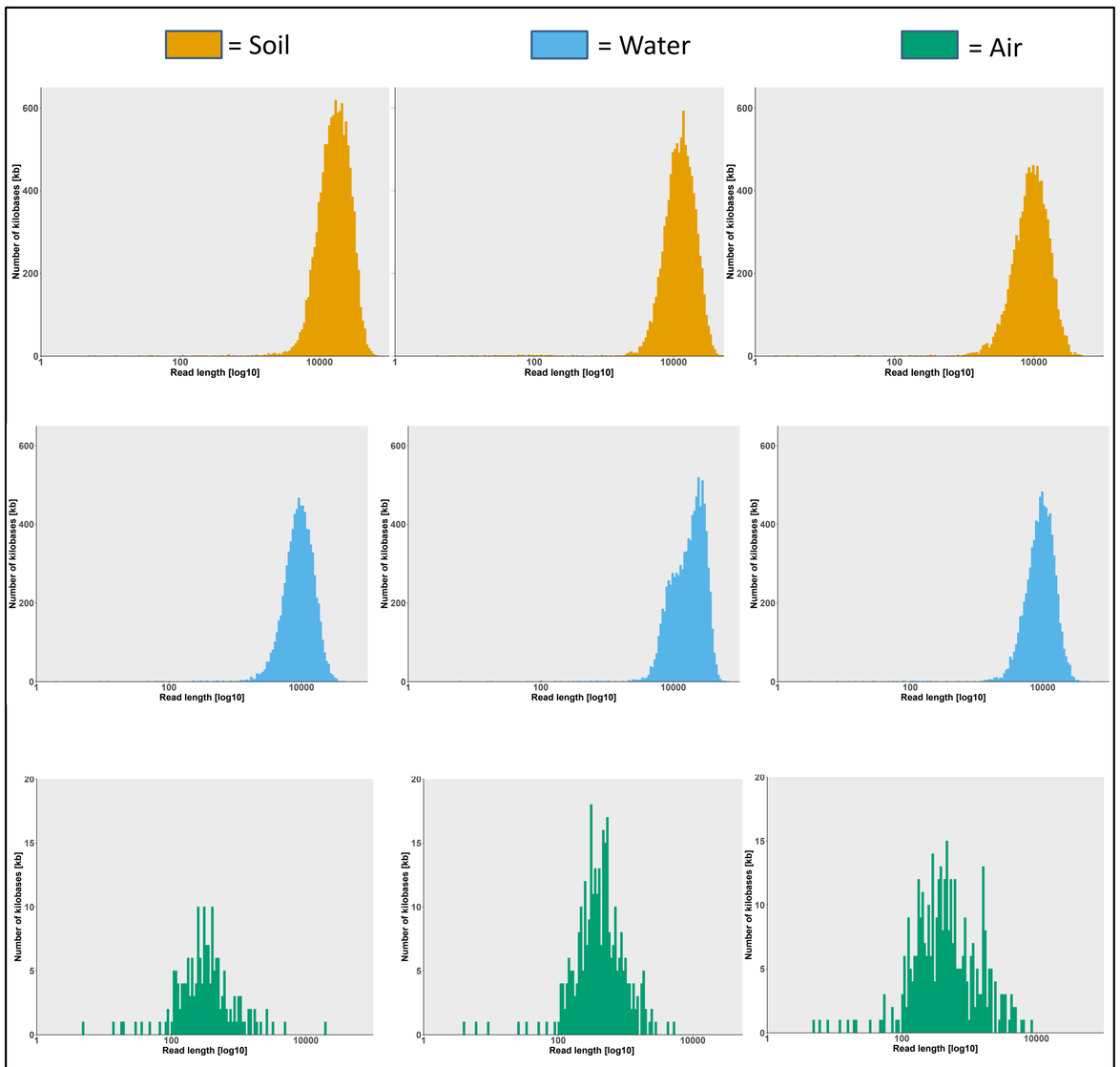
Across the world, natural systems have been degraded to an unprecedented extent, resulting in severe biodiversity loss at the microbial and macro level (*Ceballos et al., 2017*) Despite their importance for ecosystem functioning, the study of microbial diversity has obtained less attention than other taxonomic groups such as the vertebrates. Especially the **microbial diversity of air remains poorly understood** in comparison to more frequently studied aquatic and terrestrial microbiomes.

Here, we conducted a **pilot study to assess the change of microbial diversity along gradients of anthropization by using nanopore metagenomics**. We sampled three replicates of air, soil, and water at Castellbisball, an industrial area at the intersection of natural and urban environments in Spain. We processed these samples in a standardized manner using spin column DNA extraction and nanopore shotgun sequencing (RBK114-24) to subsequently perform High-Accuracy (HAC) basecalling and nanopore metagenomic studies.

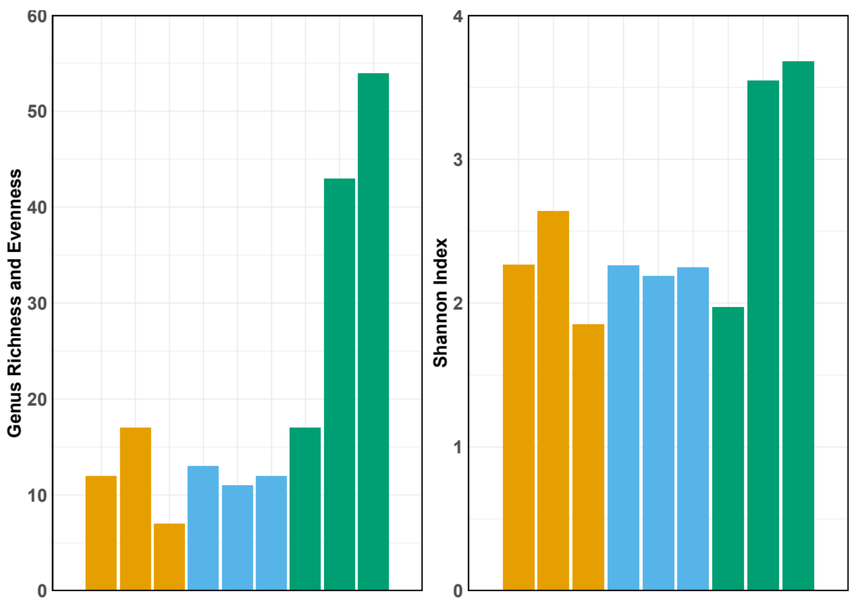




Nanopore Shotgun Sequencing



Microbial Diversity



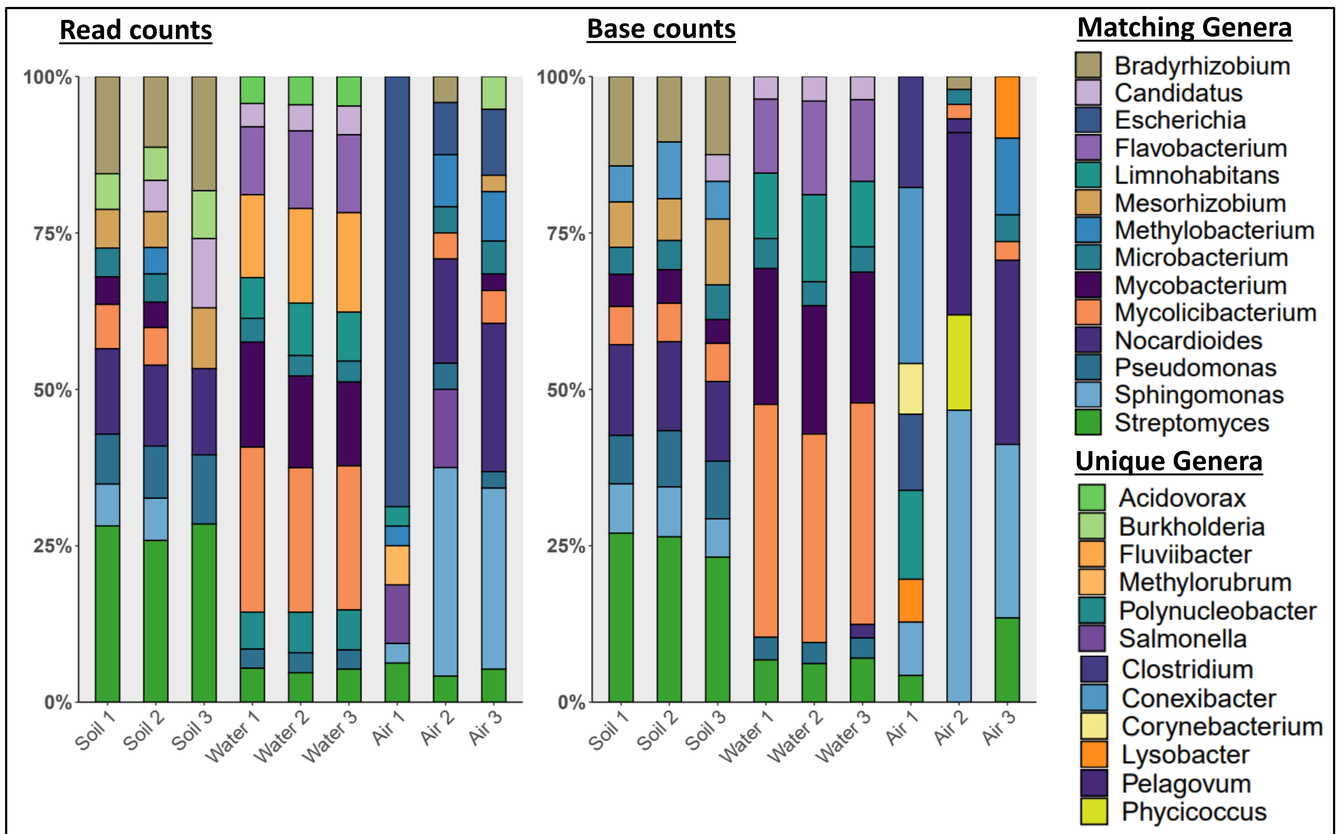
The air samples exhibit higher genus richness and evenness compared to soil and water samples, indicating ***a more diverse microbial community in the air environment (see phylogenetic tree in "Air Microbiome")****.*

Functional Annotation

Environment	Gene	Average Accuracy
Air	Mycobacterium chelonae 16S rRNA mutation conferring resistance to kanamycin A	89.5%
Air	Streptomyces cinnamoneus EF-Tu mutants conferring resistance to elfamycin	80.6%

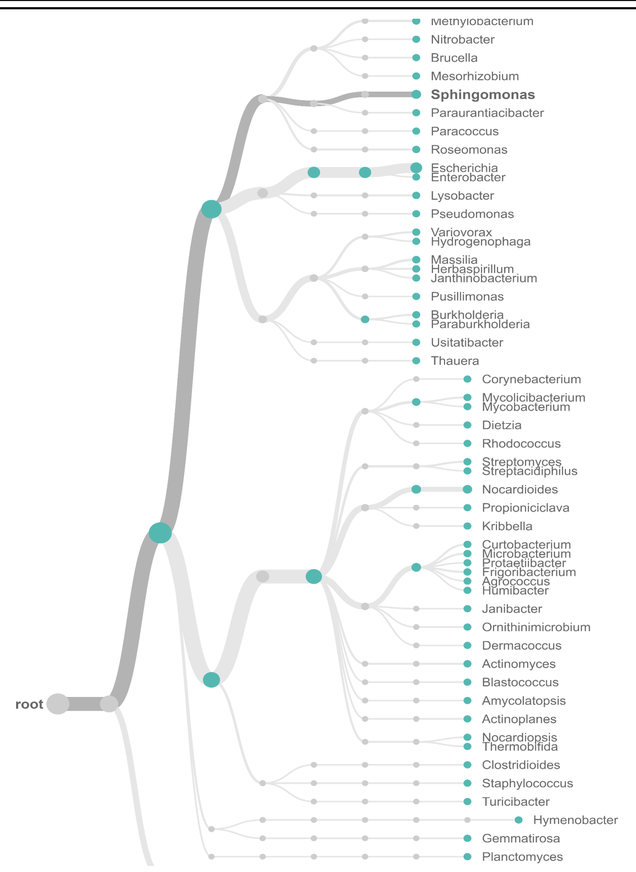
The EPI2ME anti micriobial resistance (AMR) analysis detected a Mycobacteroides chelonae-related sequence with a 16S rRNA mutation conferring resistance to kanamycin A and a Streptomyces cinnamoneus EF-Tu mutant resistant to elfamycin in a low biomass metagenomic air sample. These resistances could reduce the efficacy of antibiotic treatments and increase infection risks for humans, highlighting the need for monitoring and mitigating the spread of antibiotic-resistant bacteria in the environment.

Nanopore Environmental Metagenomics



We performed taxonomic classification of the nanopore reads to the **microbial genus level** using Centrifuge-based What's In My Pot (WIMP) (*Kim et al., 2016).* We filtered reads for length (>50 bases) and the bacterial genera for minimum relative abundance (>1%), and then plotted the 20 genera with the highest relative abundance across all samples. We assessed the microbial composition of each sample using the relative abundance of **read counts (left) and base counts (right)**. This pilot study shows that **we can robustly assess environment-specific microbial composition**, even in inherently difficult-to-analyze air samples.

Air Microbiome



The phylogenetic tree shows a diverse taxonomic composition in our air samples. illustrates the complex relationships within the air microbiomes. This analysis highlights the value of employing nanopore metagenomic to better understand under-explored aerial microbial communities and their possible roles in ecosystems.

Outlook

Our pilot study confirms the successful application of nanopore metagenomics for the analysis of water, air, and soil microbiomes. Nanopore sequencing will therefore allow us to assess if **the environmental microbiome can act as a sentinel of anthropogenic pressures** and can be leveraged for non-invasive diagnostics of human impacts on ecosystems.

References

1) CEBALLOS G, EHRLICH PR, DIRZO R. (2017) Biological annihilation via the ongoing sixth mass extinction signaled by vertebrate population losses and declines. Proc. Natl. Acad. Sci. USA 114: E6089-E6096]
2) KIM, D., SONG, L., BREITWIESER, F. P. & SALZBERG, S. L. 2016. Centrifuge: rapid and sensitive classification of metagenomic sequences. *Genome Res,* 26**,** 1721-1729.

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