In-depth analysis of the origin of Primary Biological Aerosol Particles (PBAPs) in a temperate forest of Leipzig

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What are bioaerosols?

- Primary Biological Aerosol Particles (PBAPs) are airborne biological components which are directly emitted from the biosphere into the atmosphere.
- They include bacteria, fungi, viruses, spores, pollen, and plant or animal debris (Després et al., 2012).
- Their dispersion is influenced by physical, meteorological, and biological factors.

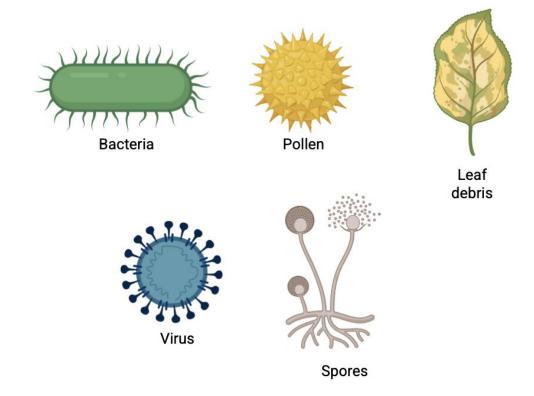


Fig 1: Different types of PBAPs (Made in BioRender)



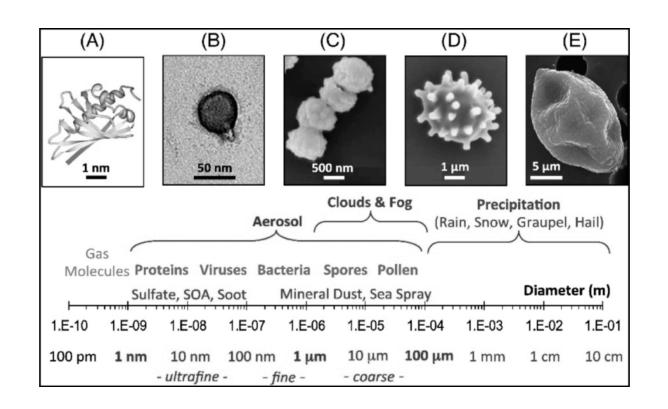


Fig 2: Characteristic size ranges of atmospheric particles and bioaerosols

(A) protein (B) virus, (C) bacteria, (D) fungal spore and (E) pollen grain

(Fröhlich-Nowoisky et al. 2016)

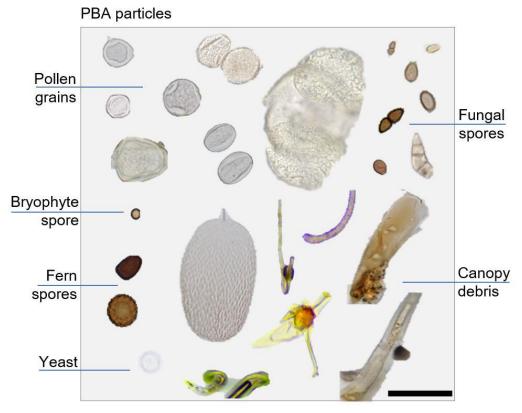
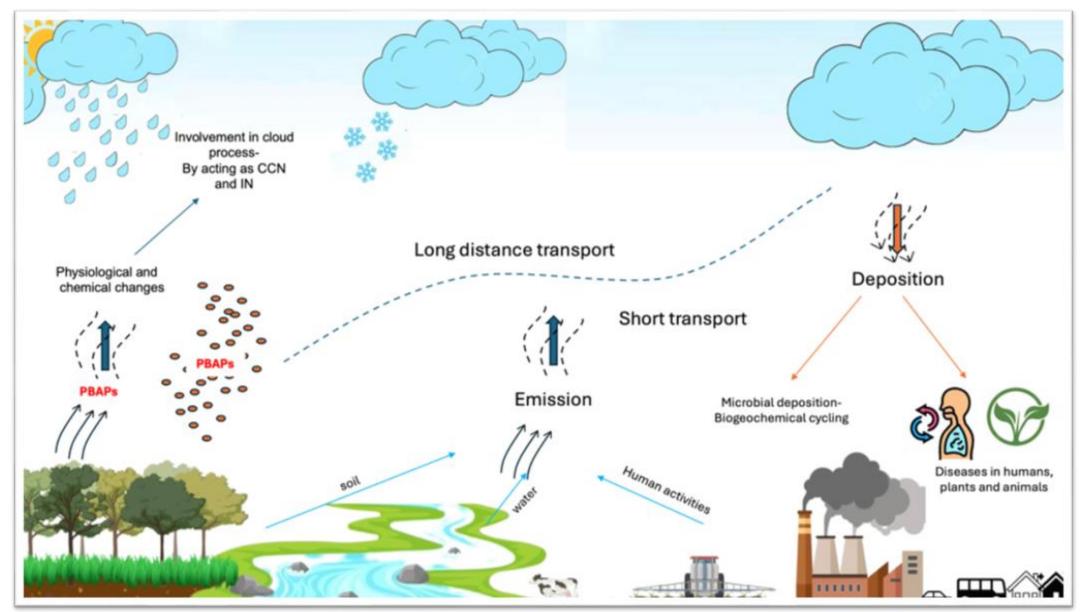


Fig 3: Microscopic images of giant aerosol particles found at ATTO (Barbosa et al., 2022)

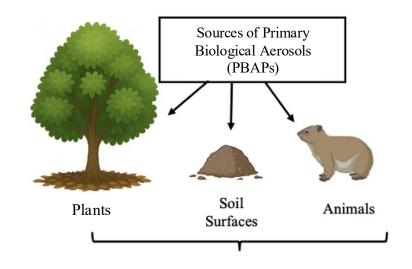


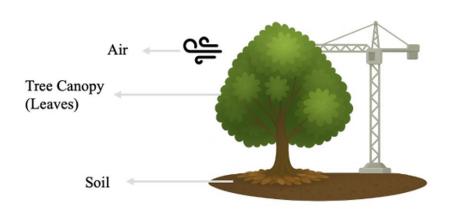
Why to study them?



RESEARCH OBJECTIVE-

STUDY SITE- Leipzig Canopy Crane (LCC)





STUDY MICROORGANISMS-



SEASONAL COMPARISON-



CHALLENGE-



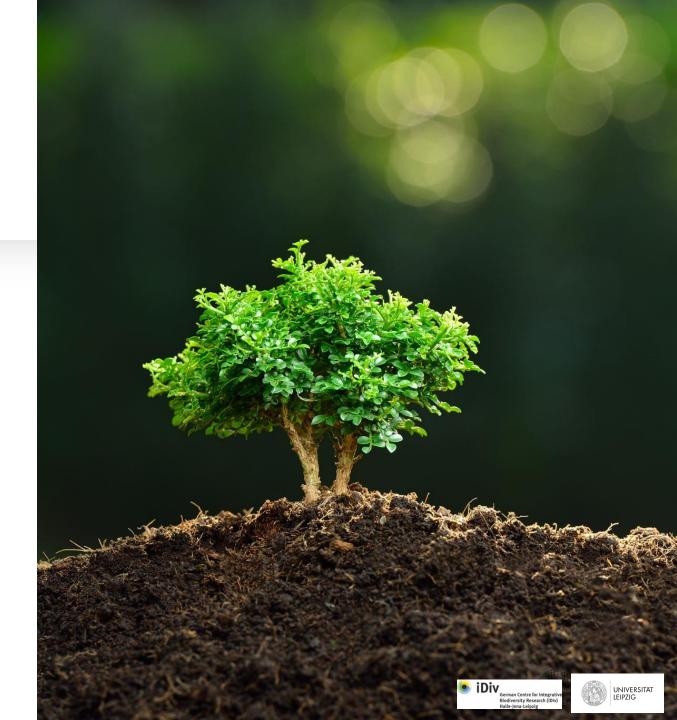
PBAPs travel short as well as long distances making it difficult to trace their exact sources.

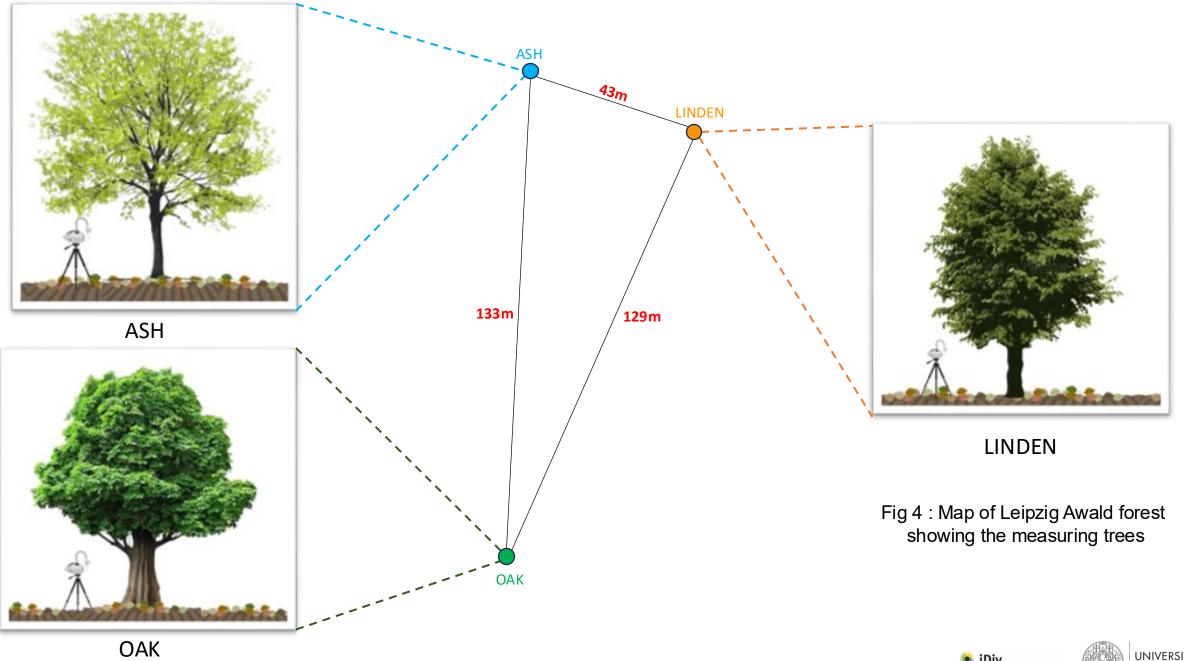


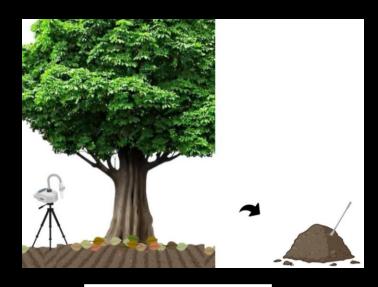


Objectives: Tracing the origins of PBAPs

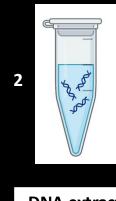
- To compare air vs. soil microbial diversity and composition.
- To determine the extent to which soil acts as a source of airborne microbes.
- To evaluate the influence of seasonality and tree type on PBAPs diversity.
- To advance the understanding of ecological role of the PBAPs contributing to broader insights into biodiversity dynamics and ecosystem functioning.



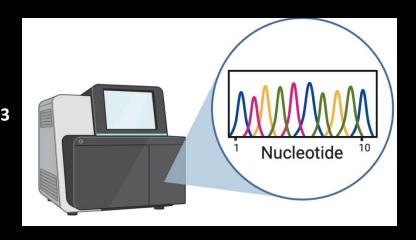




Air and Soil sampling



DNA extraction



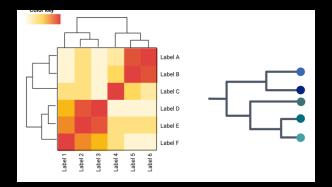
Next Generation Sequencing (NGS)

qPCR

Flow cytometry

Organic matter analysis

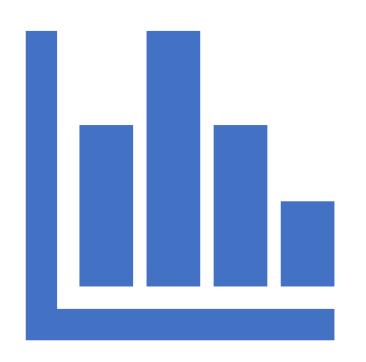
METHODOLOGY



Amplicon Sequence Variance (ASV) analysis







Results

- 1. Alpha Diversity
- 2. Beta Diversity
- 3. Taxonomic classification





BACTERIA FUNGI

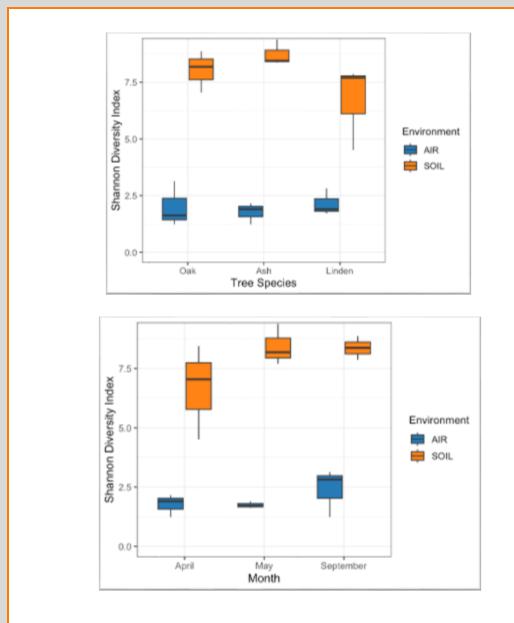


Fig 5: Alpha diversity-Shannon index for bacterial samples i) based on tree type ii) based on month

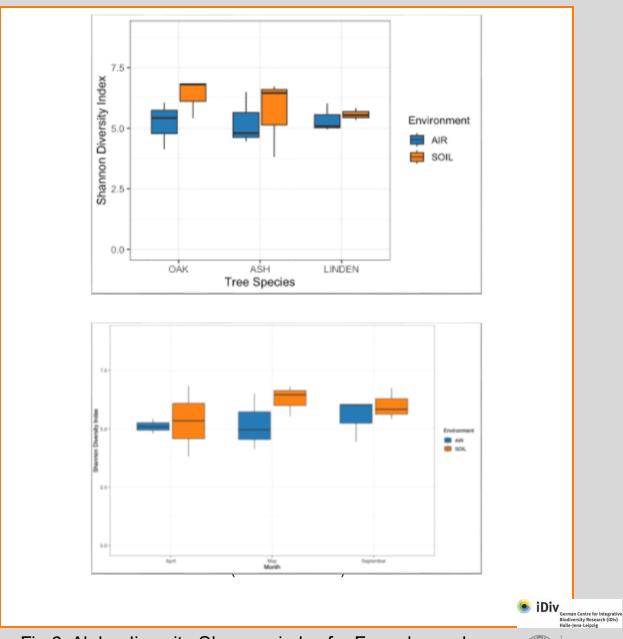


Fig 6: Alpha diversity-Shannon index for Fungal samples i) based on tree type ii) based on month



BACTERIA FUNGI

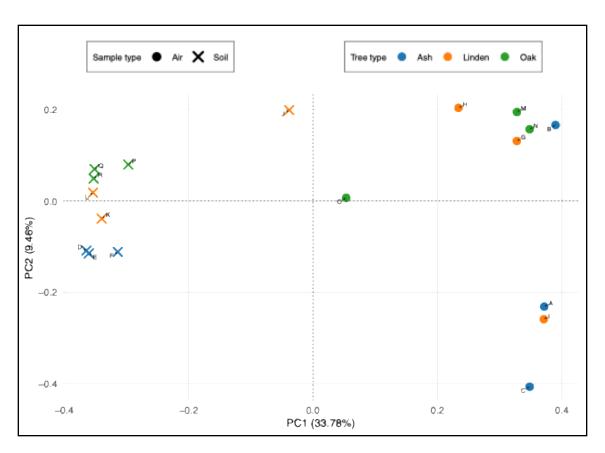


Fig 7: Beta diversity-Unweighted UniFrac for bacteria (based on tree type and month)

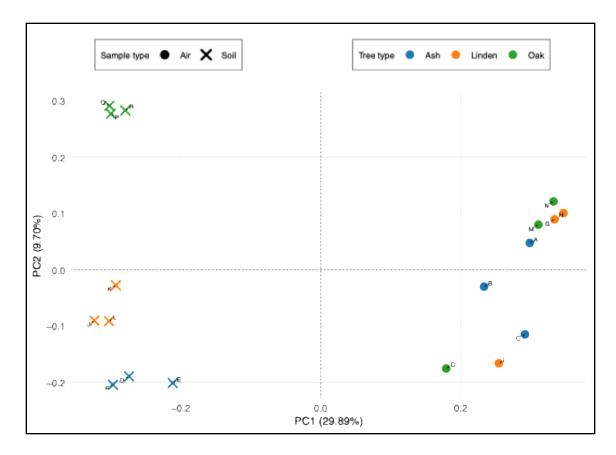


Fig 8: Beta diversity-Unweighted UniFrac for fungi (based on tree type and month)



BACTERIA

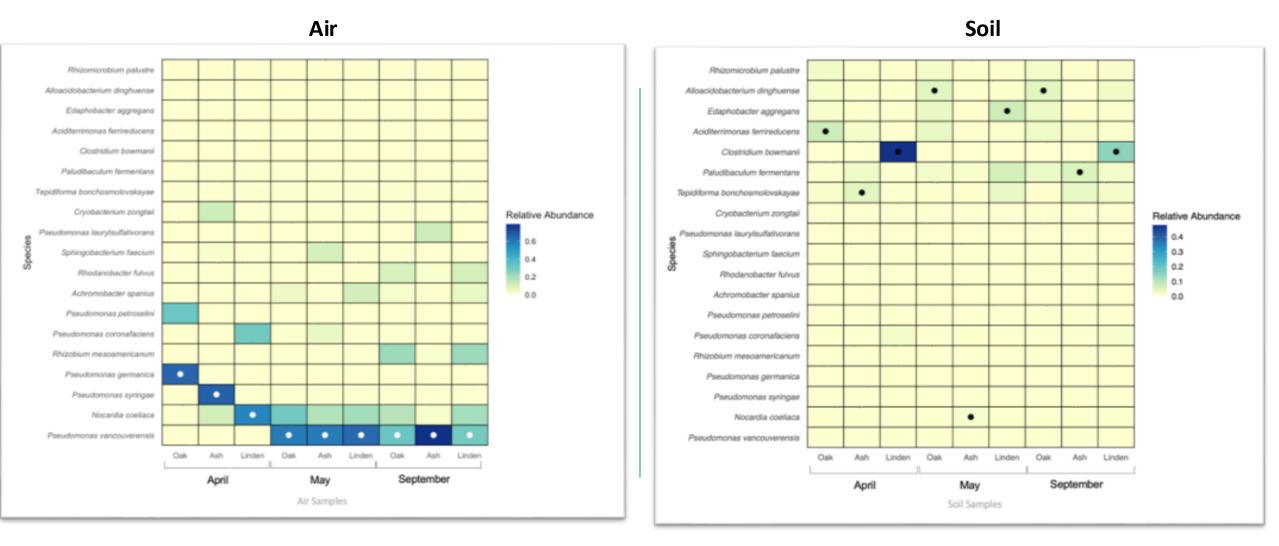


Fig 9: Heat Map comparing most dominant bacterial species





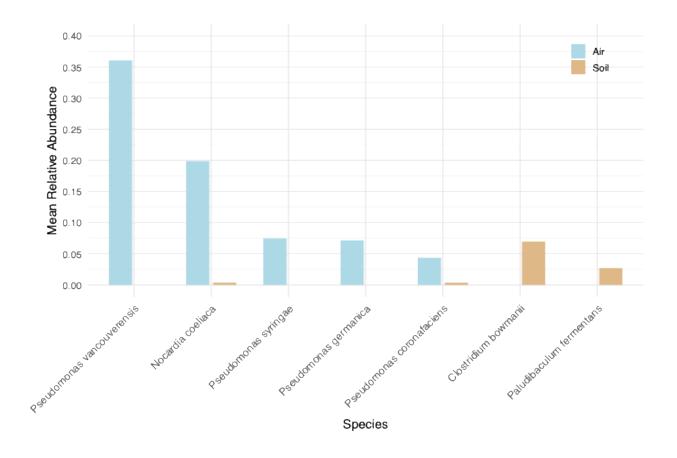


Fig 10: Comparison of mean relative abundance of the most dominant bacterial species in the air samples





FUNGI

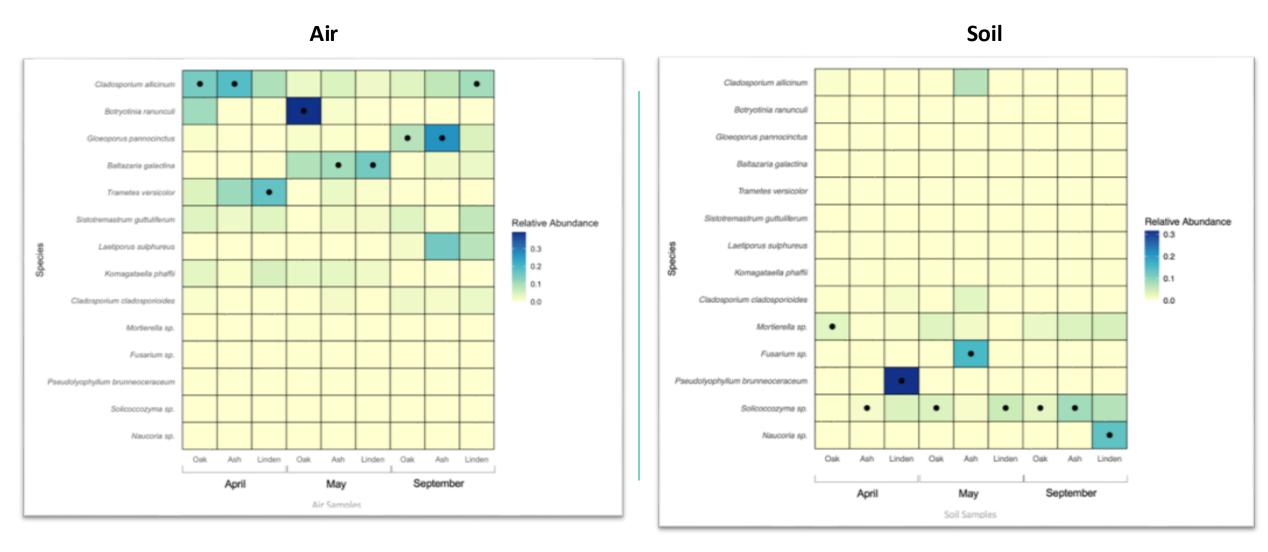


Fig 11: Heat Map comparing most dominant fungal species





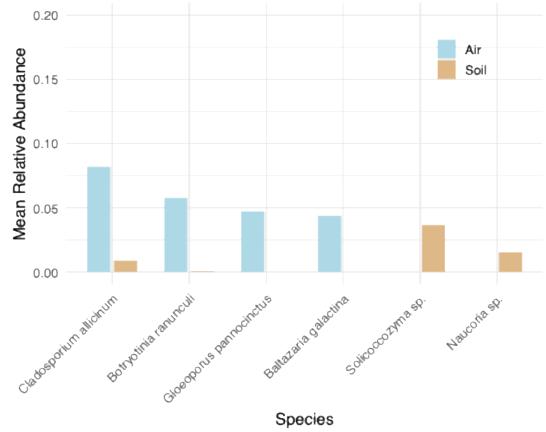


Fig 11: Comparison of mean relative abundance of the most dominant Fungal species in the air samples



FUNGuild based classification

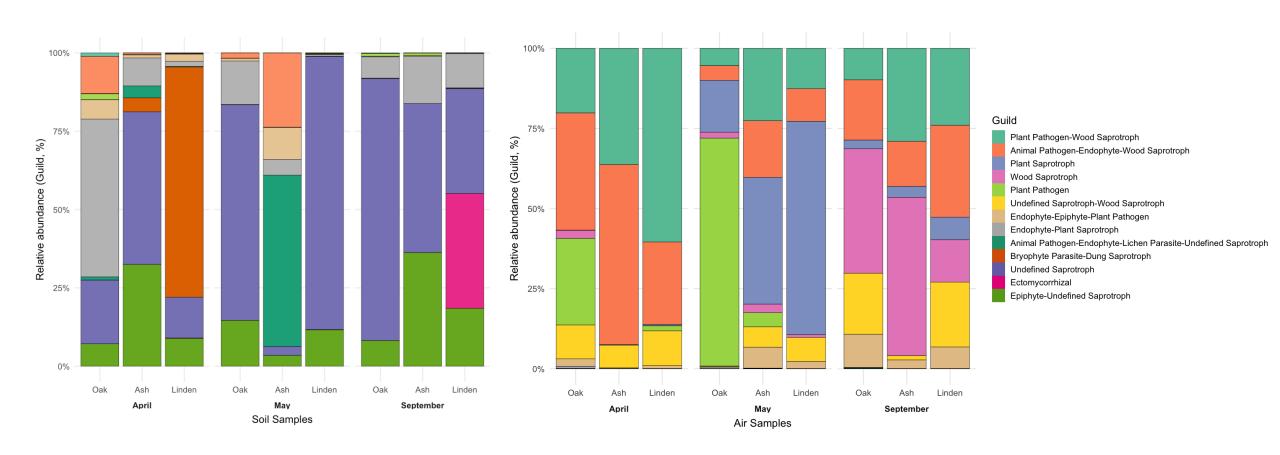


Fig 13: Comparison of to most dominant guilds in air a) Soil and b) air samples





Conclusions:

- For Bacteria, air samples showed lower diversity, mostly dominated by a few similar species like Pseudomonas spp. while soil samples were more diverse and even.
- For Fungi, air samples showed relatively higher diversity, similar to the corresponding soil samples.
- Microbial communities in soil and air were distinctly separated in composition.
- Seasonality and tree type did not seem to influence the composition and diversity of PBAPs in our dataset.
- Soil does not appear to be the original source of the dominant airborne microbes, suggesting the need for further exploration of other sources like phyllosphere and surrounding water bodies.



- For the ecological role analysis of Fungi, the soil samples showed dominance of saprotrophs and epiphytes while the air samples showed dominance of wood saprotrophs and plant/animalpathogens.
- The presence of potential plant as well as animal pathogens in the air reinforces the importance of air monitoring.

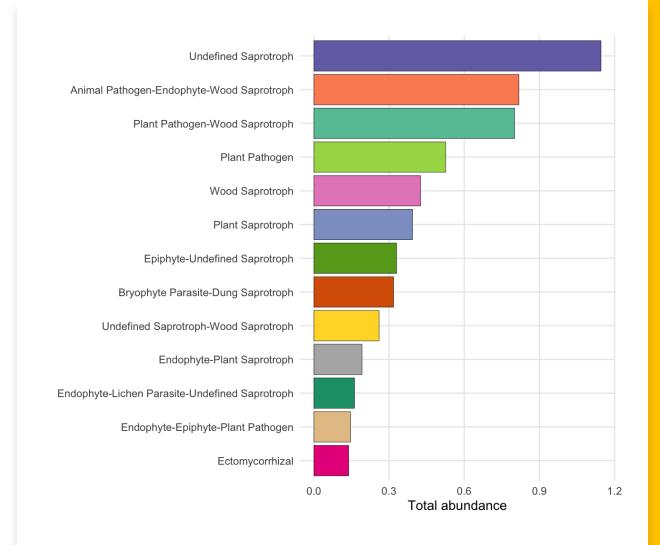


Fig 14: Most dominant guilds in soil and air samples





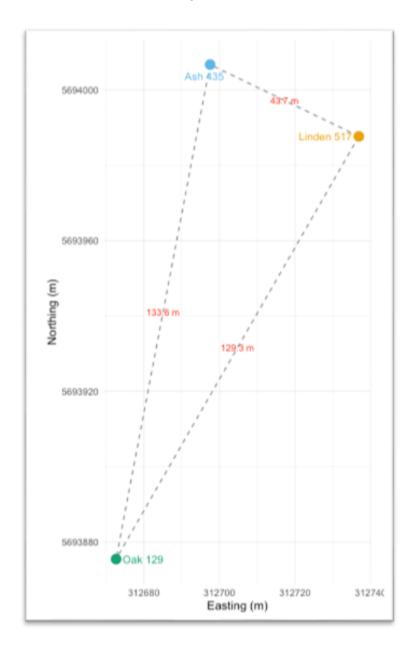




THANK YOU!



Supplementary material-



| | Tree | Sample type | Month |
|---|------|----------------|-----------|
| Α | Ash | Air | April |
| В | Ash | Air | May |
| С | Ash | Air | September |
| D | Ash | Soil | April |
| Ε | Ash | Soil | May |
| F | Ash | Soil | September |

| | Tree | Sample type | Month |
|---|--------|----------------|-----------|
| G | Linden | Air | April |
| Н | Linden | Air | May |
| 1 | Linden | Air | September |
| J | Linden | Soil | April |
| K | Linden | Soil | May |
| L | Linden | Soil | September |

| | Tree | Sample type | Month |
|---|------|----------------|-----------|
| М | Oak | Air | April |
| N | Oak | Air | May |
| 0 | Oak | Air | September |
| Р | Oak | Soil | April |
| Q | Oak | Soil | May |
| R | Oak | Soil | September |





For 16S

• **Primers-** 16S V3-V5 region (459 bp approx)

341F: CCTACGGGNGGCWGCAG

799R: CYAACGAGCGCAACCC

- Sequencing depth: Between ~100k–120k usable reads per sample (after QC).
- Amplicon size: ~460 bp (16S V3–V5 region, 341F, 799R).
- **Primers**: Standard 341F/799R primer set, removed during preprocessing.
- Bacteria (16S V3–V5, 341F–799R primers)
- Databases: NCBI NT + RDP
- Reads per sample (raw to non-chimeric):

Raw data (initial reads): ~125,099

Non-chimeric reads (after removing chimeras): ~36,837

ASVs after length filtering (final usable reads): ~35,067





For ITSFngs

- The fungal sequencing targeted the ITS region (Internal Transcribed Spacer).
- Primers- ITS1F (forward) and ITS2R (reverse).
 - ITS1F: CTTGGTCATTTAGAGGAAGTAA
 - ITS2 R: GCTGCGTTCTTCATCGATGC
- Amplicon size: 250–600 bp (variable across fungal taxa).
- Databases: UNITE Fungi + NCBI NT + UNITE INSD All eukaryotes.
- Analysis: ASV (DADA2) pipeline
- Reads per sample (raw to non-chimeric):

Raw: 102,544 – 151,989 (median 134,596)

Non-chimeric: 84,494 – 118,802 (median 110,264,

mean ~107,930)

Overall retained (final vs. raw): ~82.7%





| Month | Mean temperature (average) | RH% (average) | Wind speed (average) |
|----------------|-------------------------------|---------------|----------------------|
| April 2024 | ≈ 11.3 °C | 72 % | 14.3 km/h |
| May 2024 | ≈ 15.9°C | 75 % | 15 km/h |
| September 2024 | ≈ 13°C | 69 % | 21.1 km/h |



