B42: URBAN PHYLLOSPHERE MICROBIOMES AS AGENTS OF AIR POLLUTANT BIODEGRADATION: A CASE STUDY FROM MILAN, ITALY

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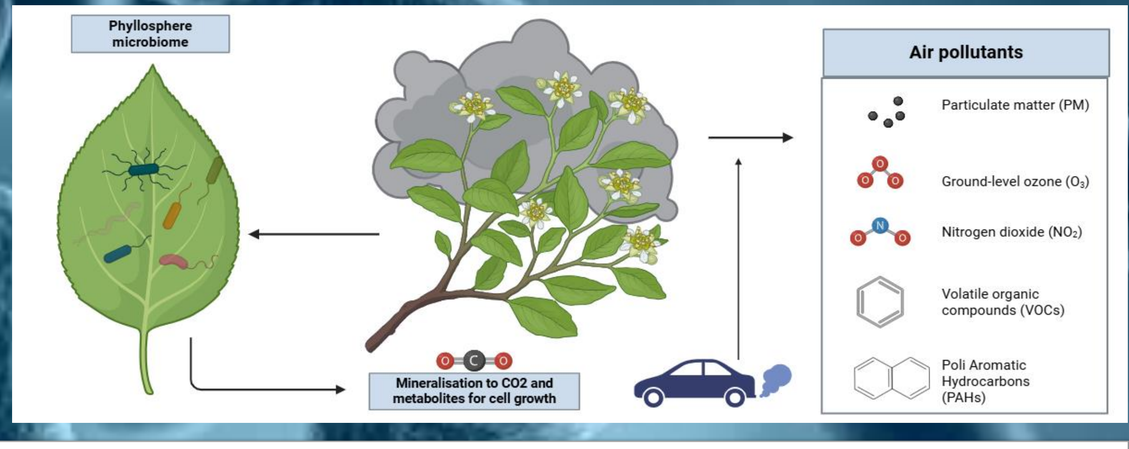
INTRODUCTION

The **phyllosphere**, which includes all aerial parts of plants, hosts diverse **microbial communities**. Each leaf can support numerous microorganisms, some of which are capable of degrading organic compounds such as hydrocarbons.

According to the World Health Organization (WHO), **air pollution** was responsible for 4.2 million premature deaths in 2019. Pollutants can be adsorbed or absorbed by leaves, where certain microbes may use them—such as naphthalene and other polyaromatic hydrocarbons (PAHs)—as sources of carbon or energy.

However, most **environmental factors** shaping these bacterial communities remain poorly understood, as do the identities and functions of many PAH-degrading bacteria.



EXPERIMENTAL PLAN

OBJECTIVES

What factors influence the spatial and temporal variability of the phyllosphere microbiome?
Study of bacterial and fungal communities.

What are microbial ecosystem services? Degradation of PAHs and amhelioration of urban air qualiTy.

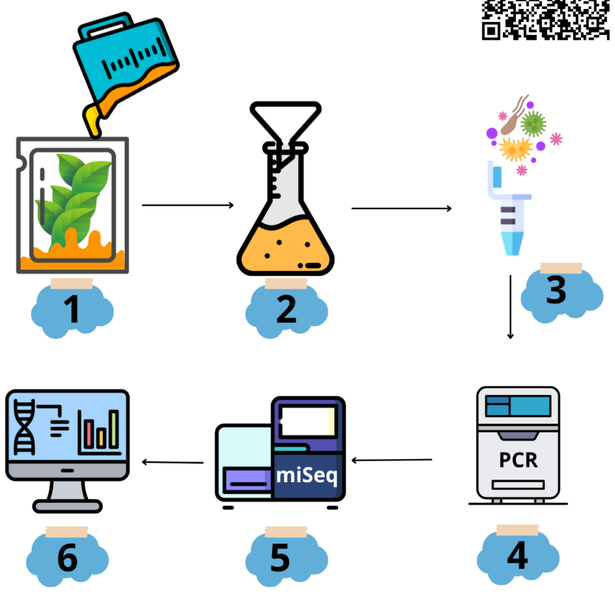
WORK IN PROGRESS

What possible applications could there be? Improve degradation of PAHs by inoculum application (Isolation of degraders, analysis through WGS and comparative genomics)

METHODOLOGY

1. Leaves-washing with a washing solution (EDTA, TrisHCl, Tween 20), sonication and agitation
2. Filtering the washing solution with a 0.45µm nitrocellulose filter
3. Performing the DNA extraction with half filter, to extract genomic DNA
4. PCR amplification using two target genes: 16S rRNA for bacteria and ITS1 for fungi
5. Metagenomic sequencing with miSeq Illumina technology on the two different amplicons
6. Bioinformatic analysis was performed to assign taxonomy using ASVs, assess α- and β-diversity, and evaluate biodegradation potential.

Our sequencing facility →



RESULTS AND DISCUSSION

BACTERIAL COMMUNITY

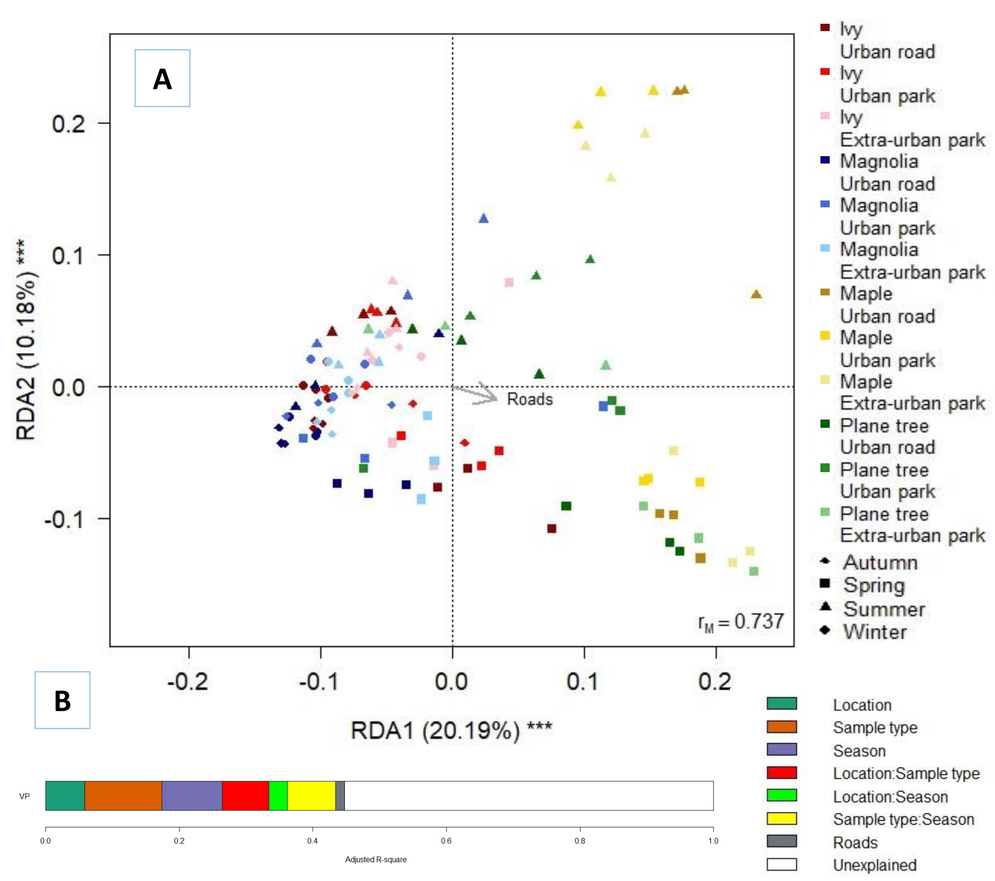


Fig 1 *RDA (**A**) and VP (Variation Partitioning)(**B**) of the bacterial community. VP shows that the model explain among 45% of the total variance associate to the data.*

- Redundancy Analysis (**RDA**) was used to relate variation in bacterial community composition to environmental variables.
- The main factors influencing community variation were **sample type** (plant species), **season** and **sampling point**. Notably, **interactions** between sample type and both season and location also contributed substantially to the explained variance.
- Among the 40 environmental variables considered, only the percentage of **roads** within a 100 m radius was statistically significant in explaining variance.

HYDROCARBONS DEGRADATION POTENTIAL

- The relative abundance of bacterial genera with hydrocarbon-degrading potential was estimated by multiplying their original abundance by a genus-specific coefficient (0–1), calculated from the proportion of genomes harboring **key degradation marker genes**, identified via HMM searches against the GTDB database.
- The data indicate two main types of hydrocarbon metabolism: **aerobic alkane degradation and degradation of mono- and poly-aromatic hydrocarbons**, with highest potential in spring—especially in maple and plane tree samples from the extra-urban park and in ivy from high-traffic urban roads—followed by a decline in summer and autumn.

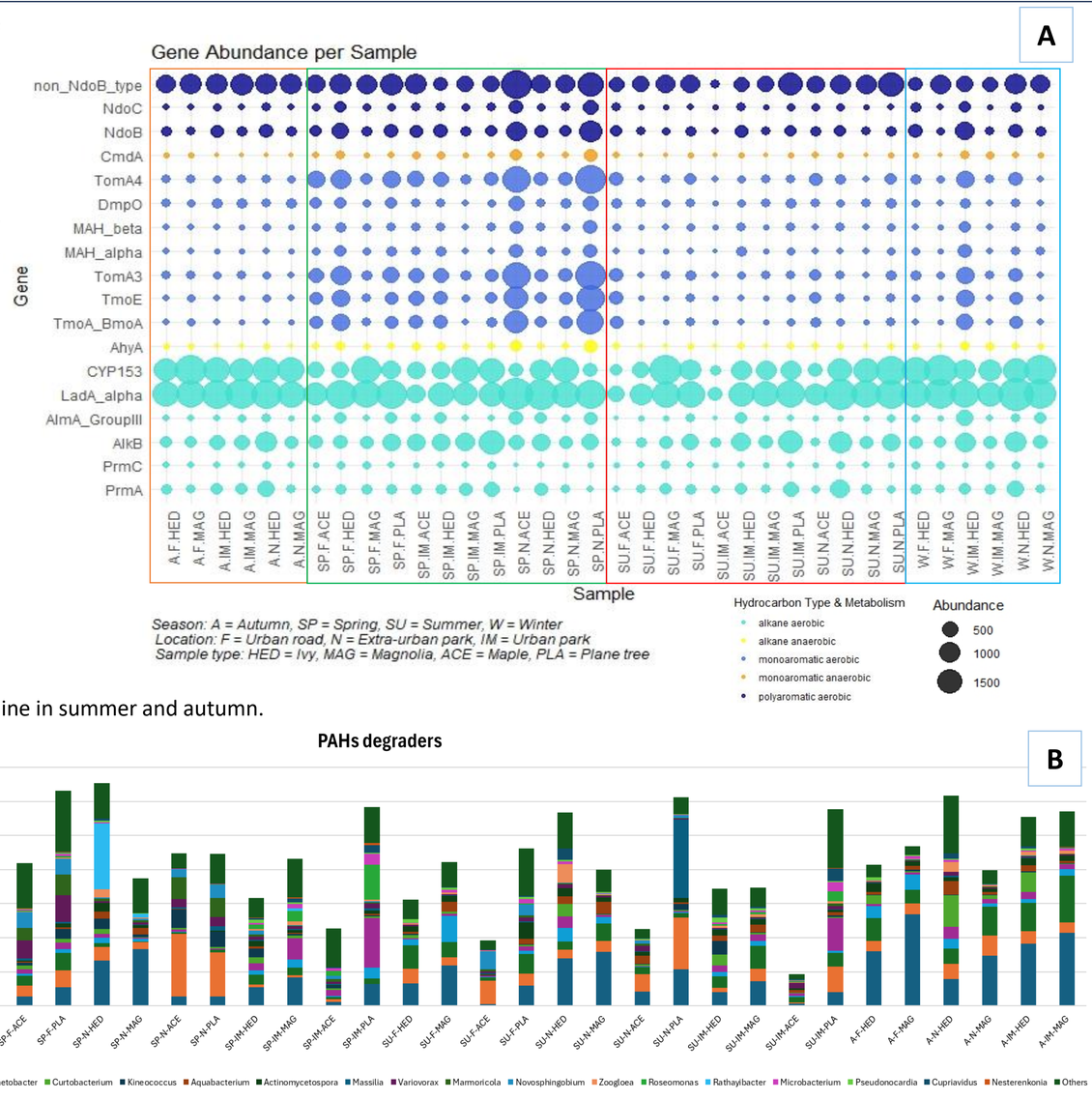


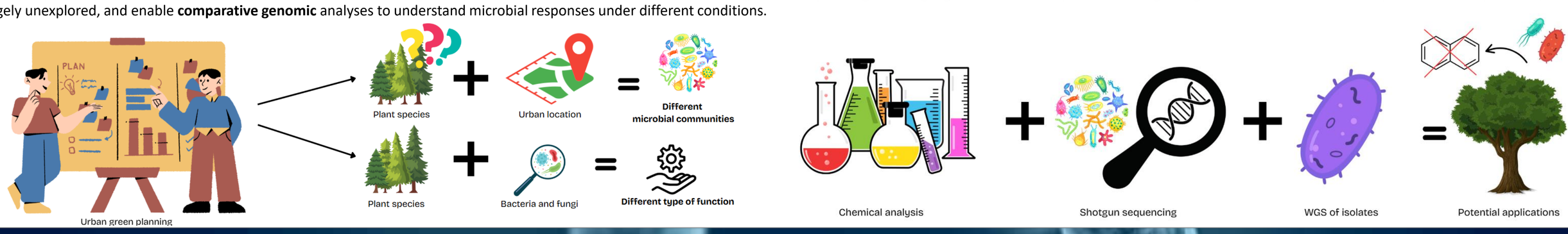
Fig 2 *Bubble plot (**A**) representing the relative normalised abundance (10000) of each sample multiplied by each coefficient from the HMM models, and (**B**) bar plot indicating the most abundant genera for PAH degradation.*

CONCLUSIONS AND FUTURE PERSPECTIVE

This study identified the main factors shaping bacterial and fungal communities. The activity of PAH-degrading microbes was highest in spring, likely due to airborne pollutants deposited on leaves after winter, when heating systems are most active.

To better understand microbial community shifts, **chemical analyses** of both leaves and airborne particles are needed, as 60% of the variance remains unexplained.

Future steps will include **isolating microorganisms** from leaves through enrichment on various PAHs, followed by whole-genome sequencing. Building a **genome bank** of hydrocarbon-degrading or -resistant isolates will help uncover their potential, still largely unexplored, and enable **comparative genomic** analyses to understand microbial responses under different conditions.



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