HNEC-ALIs were infected with the human coronavirus (hCoV) 229E, and infectious virus particle production (Plaque assay) as well as viral genomes (dPCR) were assessed after 48 and 72h.

Results: The commonly used pollen extracts resulted in the release of cytokines into different compartments than exposure to the more physiological pollen aerosols. The response to birch pollen aerosol was characterized by an early (24h) induction of IL-8, IL-6, IL-1b, IL-18 and IL-25, and a late (48h) release of TNF-a and CCL22. Exposure to grass- and ragweed pollen resulted in a stronger overall cytokine response than to birch pollen, with a significantly stronger release of IL-1 family cytokines and IL-25. hCoV 229E infection at 0.25 MOI led to a rapid (24h) pro-inflammatory cytokine production mainly on the apical side, whereas type I or type III interferons were not produced at any time.

Outlook: In a co-exposure model, pollen pre-exposure will be combined with subsequent virus infection, and cytokine response and virus replication will be compared with virus infection in the absence of pollen pre-exposure. In addition, another infection model with hCoV NL63 will be established to further investigate the immune response under co-exposure to coronaviruses and birch pollen.

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Airborne Environmental DNA and the Unraveling of Terrestrial Plant Biodiversity

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Abstract

Alpine plant communities are crucial hotspots acting as open-field laboratories for studying the effects of climate change on plant composition, productivity, and phenology. Estimating plant biodiversity over large areas, though, can be challenging due to the labor-intensive efforts required for surveys carried out by traditional methodologies.

The ALPoll project aims to investigate the role of bioaerosols in the estimation of alpine biodiversity, including the informative potential of DNA.

In order to design optimized protocols to analyze environmental DNA (eDNA) from gravimetric aerobiological sampling, a preliminary study was conducted in a rural site, on a set of 13 weekly specimens, collected in duplicate, by a Sigma-2 gravitational sampler of airborne particles. eDNA was extracted with Qiagen DNeasy Plant Mini Kit and ITS1 and ITS2 barcodes were

selected for the DNA amplification, targeting the sequences with diverse primer pairs. Different enzymes for the amplification (GoTaq® and KAPA HiFi DNA polymerases) were tested. Illumina sequencing was performed and an efficient bioinformatic pipeline was designed to improve raw data analysis and precision and efficiency of the taxonomic assignments. Starting from Banchi et al. (doi.org/10.1093/database/baz155) and Quaresma et al. (doi.org/10.1038/s41597-024-02962-5) and intersecting the plant species list for the Italian Alps of Trentino (La Flora del Trentino, Prosser et al., 2019), a curated reference database was built. Sample replicates were also analyzed by optical microscopy for the comparison of data.

Results report the taxonomic resolution of both techniques and seasonal changes in the biodiversity of aerobiome, together with quantitative estimates of airborne biological particles for plant taxon. The number of Operational Taxonomic Units (OTUs) produced by the raw sequences, as well as their taxonomic classification referred to the reference database, differs depending on selected barcodes (ITS1 and ITS2), primer pairs and polymerases, used in the DNA amplification process. In particular, ITS1 barcode generates the higher number of OTUs (858 vs 787 of the best ITS2 combination of primer pairs and polymerase), but the higher number of plant taxa is classified after the ITS2 sequencing (45 families, 82 genera and 50 species). The results are consistent with ITS1 being more effective for fungi taxonomic assignment, and ITS2 maximizing plant classification, thus potentially complementing each other. The comparison of microscopy and metabarcoding data shows a high degree of correlation for many taxa (e.g. Poaceae, Pinaceae or Salicaceae). In addition, the accuracy of classification achieved by ITS1 or ITS2 results is in the vast majority of cases more specific than classification by microscopy.

The final contribution of this study will allow a definition of the different informative potential and complementarity of the two techniques and to obtain an optimized protocol for eDNA metabarcoding to be implemented in the ALPoll project.

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