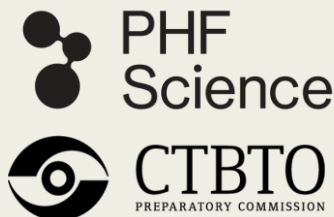


# Blowing on the Wind: eDNA Profiles from CTBTO Air Filters to Monitor Biodiversity

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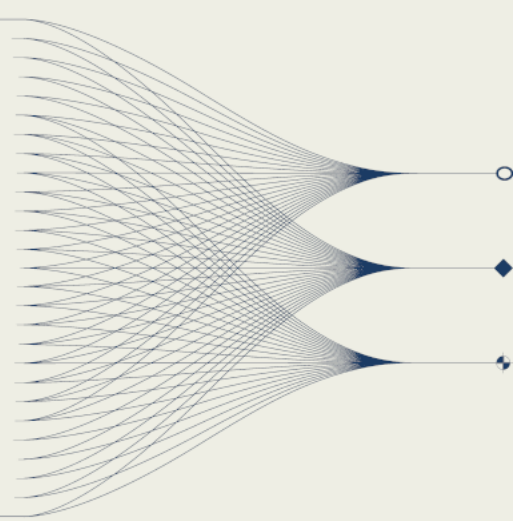
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## INTRODUCTION AND MAIN RESULTS

The CTBTO maintains a global network of monitoring stations with air filtering equipment to detect radionuclide signatures. These air filters also pick up plant, animal and microbial DNA that has been scattered on the wind.

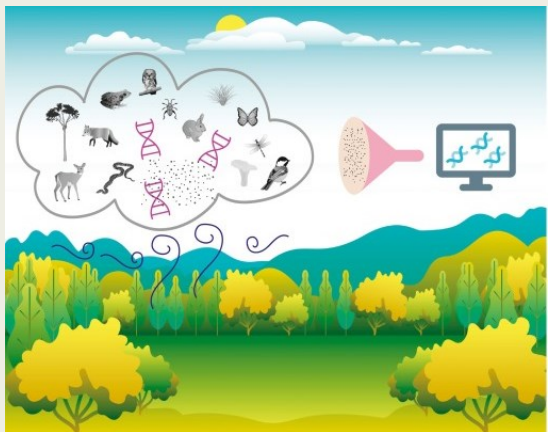
We show that this environmental DNA (eDNA) can be profiled to identify species present in the local vicinity. This provides an unparalleled opportunity to monitor global ecosystem changes and track biodiversity at an unprecedented scale.



## Introduction

The CTBT International Monitoring System (IMS) includes 80 radionuclide monitoring stations in 37 countries. At these stations, air is continuously sampled to detect radioactive particles or gases from atmospheric explosions. This is the most comprehensive air monitoring system on the planet. Filters are changed daily, compressed and, after testing, are archived at the CTBTO headquarters in Vienna.

Also collected on these air filters is the DNA of plants, animals and microbes in the local vicinity. DNA shed by species in the environment is known as environmental DNA (eDNA, Fig. 1). Characterising eDNA allows for non-invasive monitoring of species presence and abundance, and is a burgeoning and transformative tool for terrestrial biodiversity monitoring across diverse taxa.

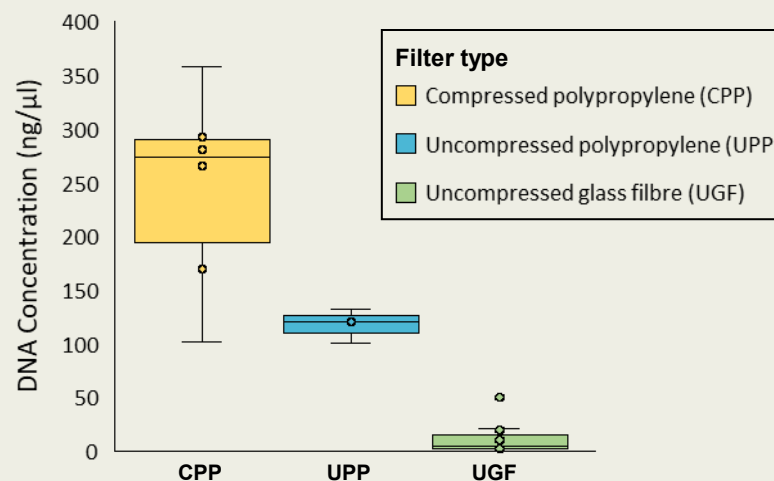


**Figure 1.** Collecting airborne eDNA allows characterisation of terrestrial biodiversity across taxonomic groups.

From: Bohmann & Lynggaard 2023. <https://doi.org/10.1016/j.j.tree.2022.11.006>

## Results

Several commercial DNA extraction kits and Taq DNA polymerases were tested on three air filter types to determine optimal lab methodology. Filter type had a strong effect of DNA recovery (Fig. 2), with highest DNA yields achieved from compressed polypropylene filters such as those used in SENYA Snow White air samplers.

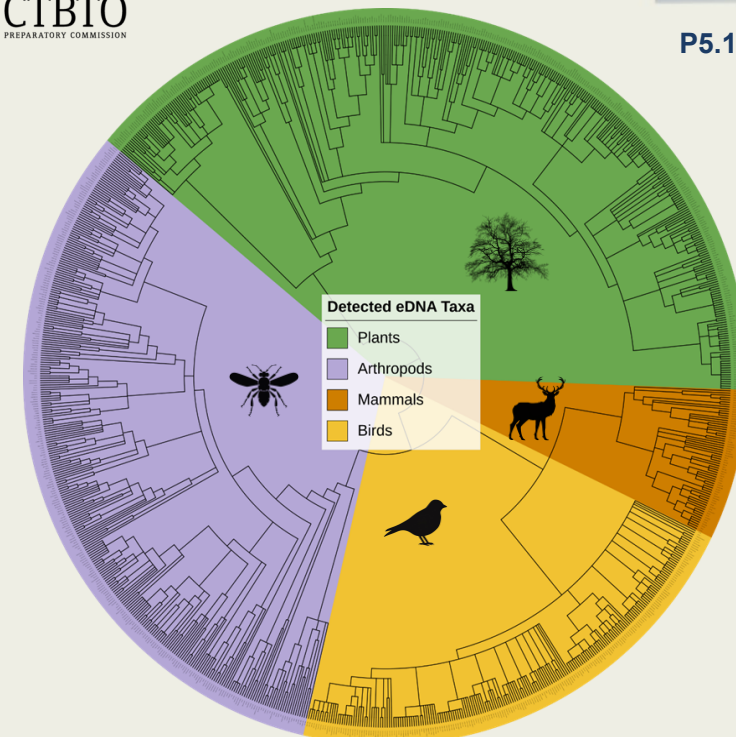


**Figure 2.** Average amount of DNA recovered (ng/μl) from three filter types (CPP, UPP and UGF) using a standard DNA extraction protocol.

DNA was PCR amplified with a selection of genetic markers and high-throughput sequenced to identify:

- Plants
- Mammals
- Birds
- Arthropods

This provided a broad perspective of the taxa collected on CTBTO air filters deployed in Vienna (Fig. 3).



**Figure 3.** Phylogeny of all species recovered from 23 air filters deployed in Vienna (rooftop of 16 floor CTBTO building) in October 2023.

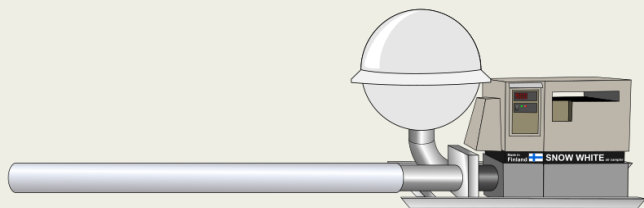
## Discussion

IMS air filters offer an unparalleled opportunity to catalogue and monitor global biodiversity over biologically meaningful timescales. The impact of events such as wildfires, deforestation and floods could also be assessed.

However, for this opportunity to be realised, agreement will need to be reached amongst state signatories (Working Group B) for rules and procedures around release of archived samples for scientific applications.

## Sample collection

SENYA “Snow White” air samplers (Fig. 4) draw air through polypropylene filters at a flow rate of approx. 20,000 m<sup>3</sup> of air per day. “Cinderella” air samples draw air through glass fibre filters, with a sample volume of approx. 12,000 m<sup>3</sup> of air per day. A network of CTBTO air samplers are deployed worldwide (Fig. 5).



**Figure 4.** SENYA JL-900 Snow White air sampler.



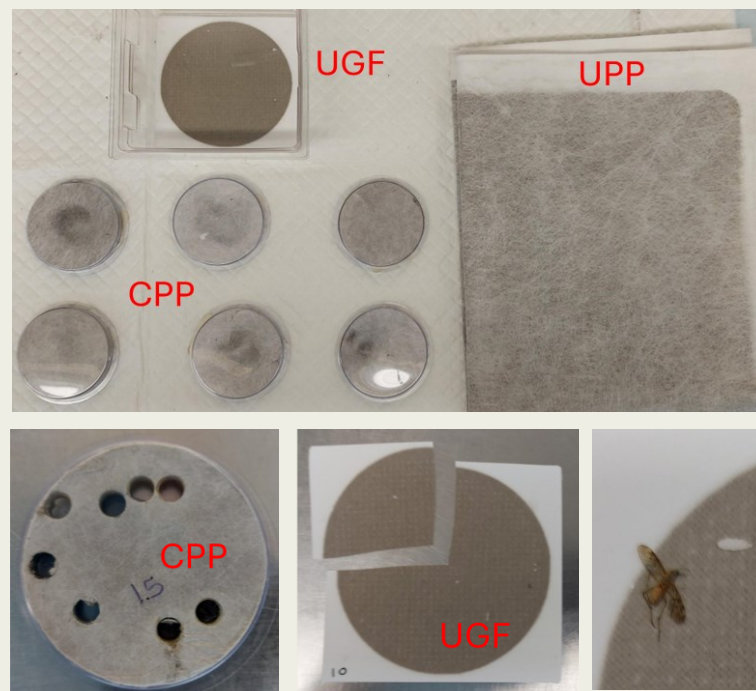
**Figure 5.** Locations of IMS CTBTO monitoring stations. Yellow dots are stations with air sampling equipment, .

## Filter types

We tested the following filter types (Fig. 6):

- compressed 3M polypropylene (CPP)
- uncompressed 3M polypropylene (UPP)
- uncompressed Whatman glass fibre (UGF)

CPP and UPP filters were collected from the rooftop, and UGF from the ground floor, of CTBTO headquarters (Vienna). Filters were 6-12 months old at the time of testing. A small subsample of each filter was collected aseptically and homogenised before DNA extraction.



**Figure 6.** Filter types tested (top panel) and subsampling of CPP and UPP filters, some of which contained macroscopic insect material (bottom panel).

## Lab methods

DNA was extracted using four commercial kits, with the Blood and Tissue Kit (Qiagen) yielding the highest DNA concentrations. Ten different genetic markers were chosen to allow recovery of invertebrate, avian, plant and vertebrate sequences to provide a broad perspective of the taxa collected on the CTBTO filters.

## Future work

The archived filters represent an exceptional biological time capsule. To realise the full potential to characterise global biodiversity changes using the eDNA collected on IMS filters, agreement amongst state signatories (Working Group B) will need to be reached on use of (subsampled) archived filters for scientific applications.



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ORIGINAL: ENGLISH

### OPERATIONAL MANUAL FOR RADIONUCLIDE MONITORING AND THE INTERNATIONAL EXCHANGE OF RADIONUCLIDE DATA

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148. After one year, the Technical Secretariat may release samples from the sample archive for verification research or other scientific research with permission of the originating country [15].

[TL Note: Discussion on sample release from archive will continue.]