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Background

Long term and high resolution historical biodiversity data are required to make accurate predictions about how biodiversity respond to human-induced alterations in the environment^{1,2}. By using high throughput DNA sequencing, we show that an unbroken line of air samples collected weekly over five decades can be used to reconstruct historical biodiversity and study the presence and temporal change in organisms in the environment.

Understanding how the biodiversity and ecosystem respond to alterations in the environment is vital for making informed decisions on biodiversity conservation and societal preparedness.

This project aims to:

- Improve the understanding how biodiversity fluctuates over time and its link to climate and environmental change.
- Study trends in pathogen distributions and produce seasonal forecasts.

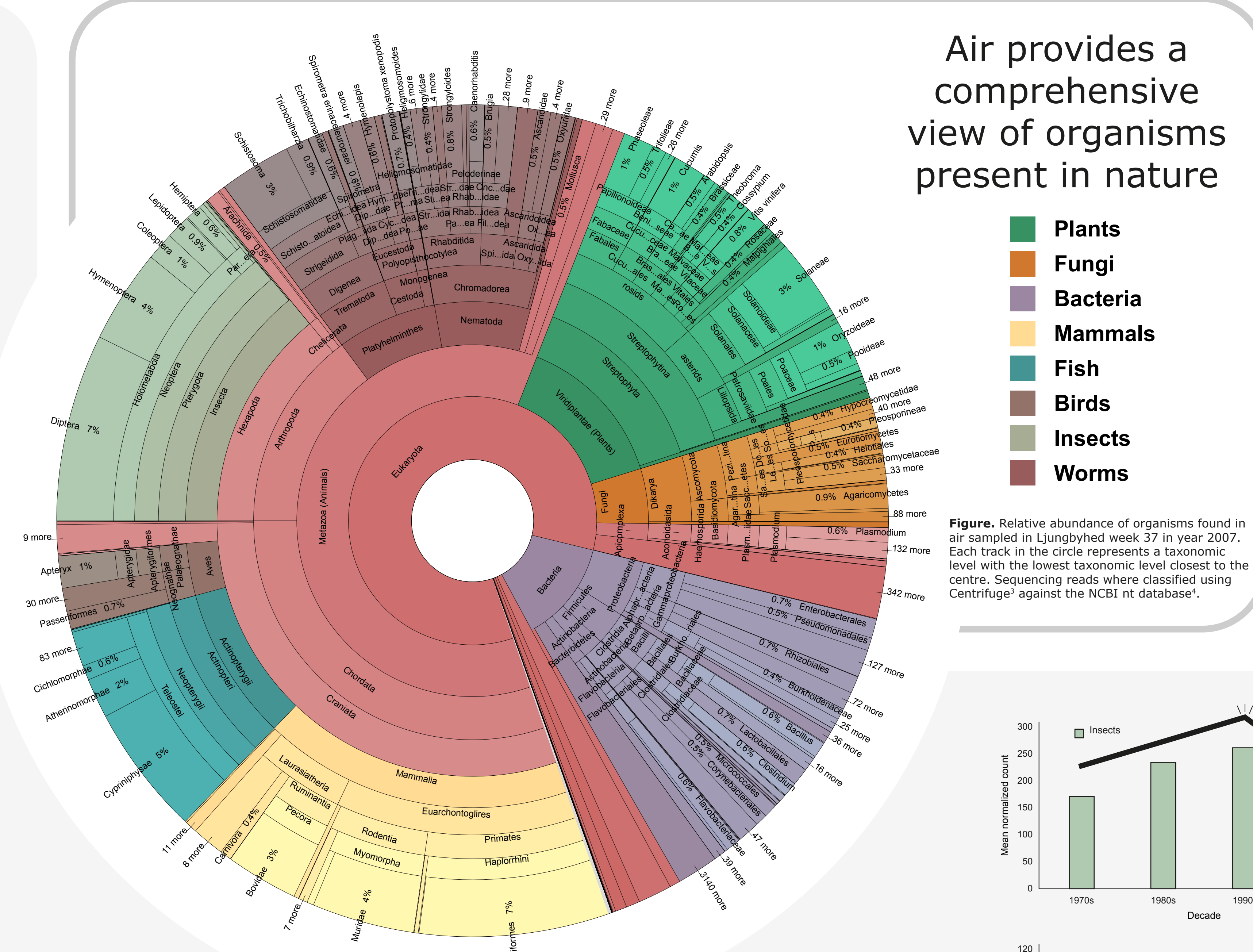
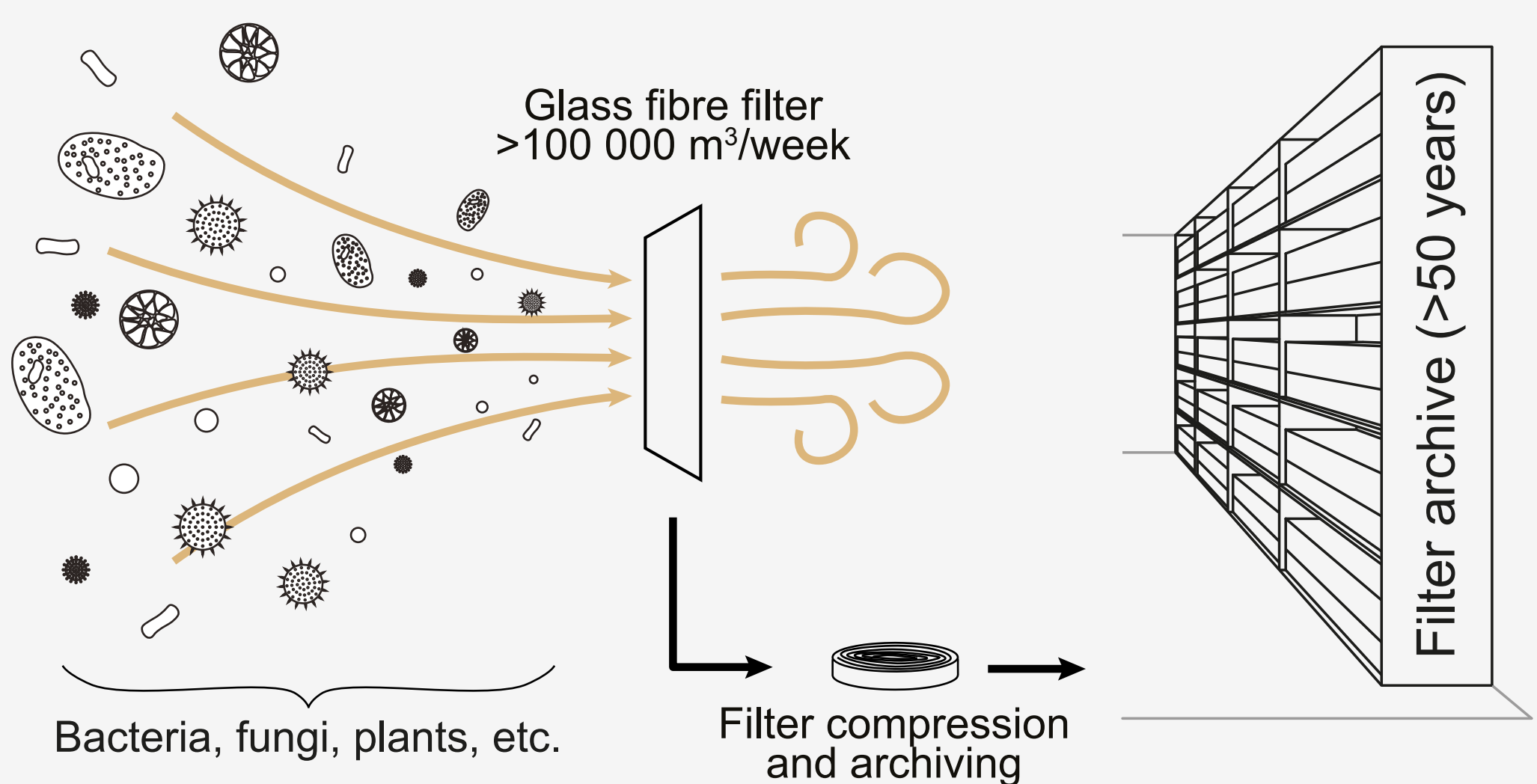
Method

Modern DNA sequencing methods allows us to sequence DNA extracted from archived air filters originally intended for radioactive fallout measurements. By matching our DNA sequences with sequence reference databases we can reconstruct the biodiversity present in the environment at the time of sampling.

Air sampling stations



Weekly air sampling



Air provides a comprehensive view of organisms present in nature

- Plants
- Fungi
- Bacteria
- Mammals
- Fish
- Birds
- Insects
- Worms

Figure. Relative abundance of organisms found in air sampled in Ljungbyhed week 37 in year 2007. Each track in the circle represents a taxonomic level with the lowest taxonomic level closest to the centre. Sequencing reads were classified using Centrifuge³ against the NCBI nt database⁴.

Historical trends in biodiversity

We have recently sequenced DNA from air filters collected in Kiruna from 1974-2008. Weekly samples from the growth season of even years (n=400) was sequenced to a depth of ~400 million paired reads (sequences) per sample.

Kiruna facts:

- Subarctic region
- Short growth season (fewer samples to sequence)
- Temperature increase during the last decades

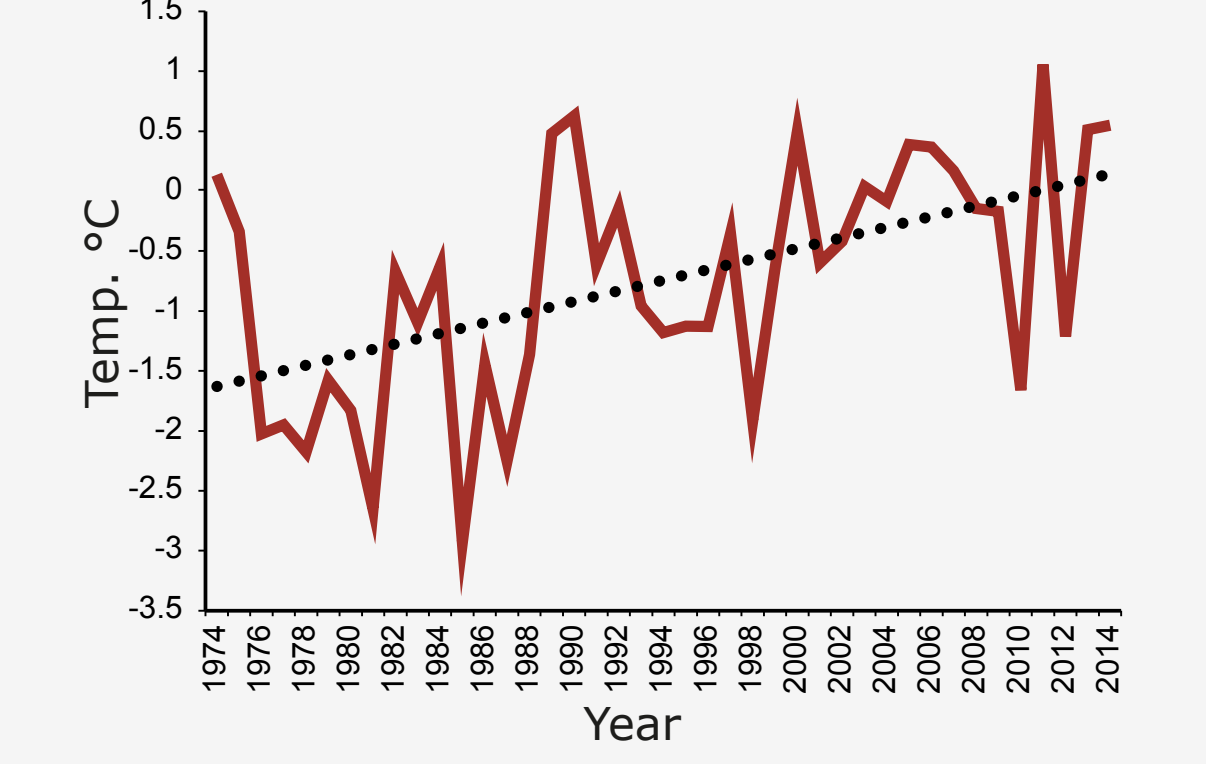


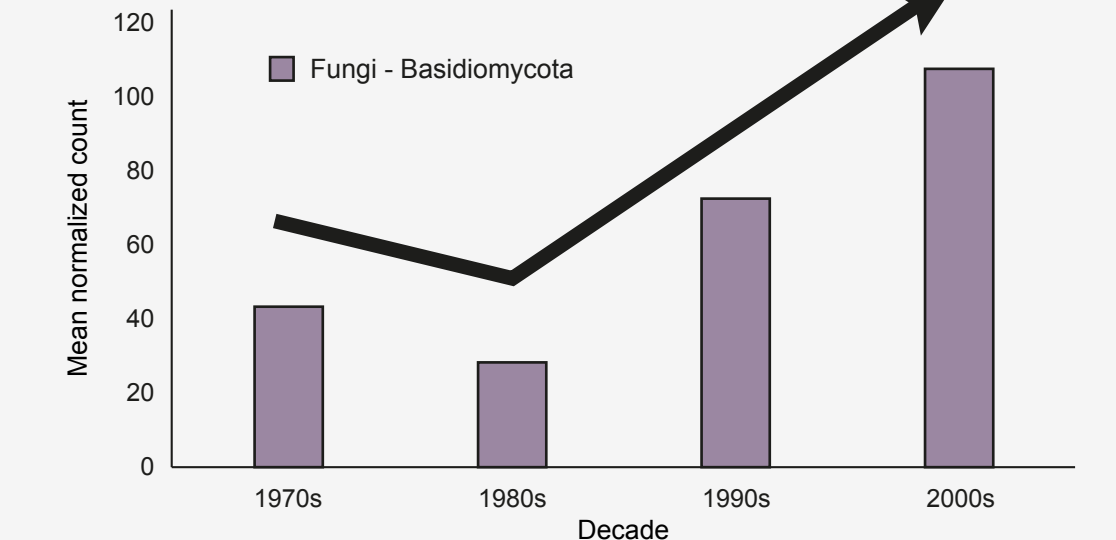
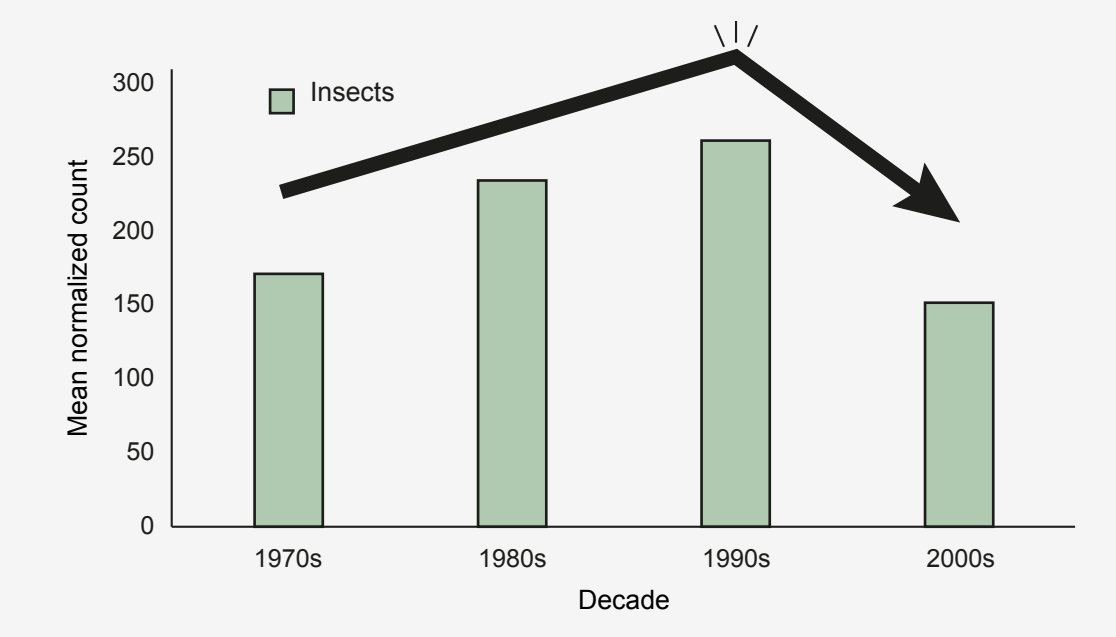
Figure. Annual average temperature in Kiruna from 1974 to 2014

Preliminary results

General trends in biodiversity

Preliminary data analysis show:

- The relative abundance of insects in Kiruna reached a tipping point in the 1990's after three decades of increase.
- The relative abundance of the fungal division Basidiomycota has steadily increased since the 1980's.



Further analysis of the data is needed to confirm these results and whether these trends are correlated with climate or environmental factors.

Pathogens

The archive allows us to study presence of pathogens in the environment e.g. the root rot causing fungus *Heterobasidion spp.*

The fungus infect spruce and cause great economic loss for the forest industry.

By studying seasonal trends in the distribution of *Heterobasidion spp.* we aim to produce seasonal abundance forecasts based on weather conditions, which can improve countermeasure effectiveness.

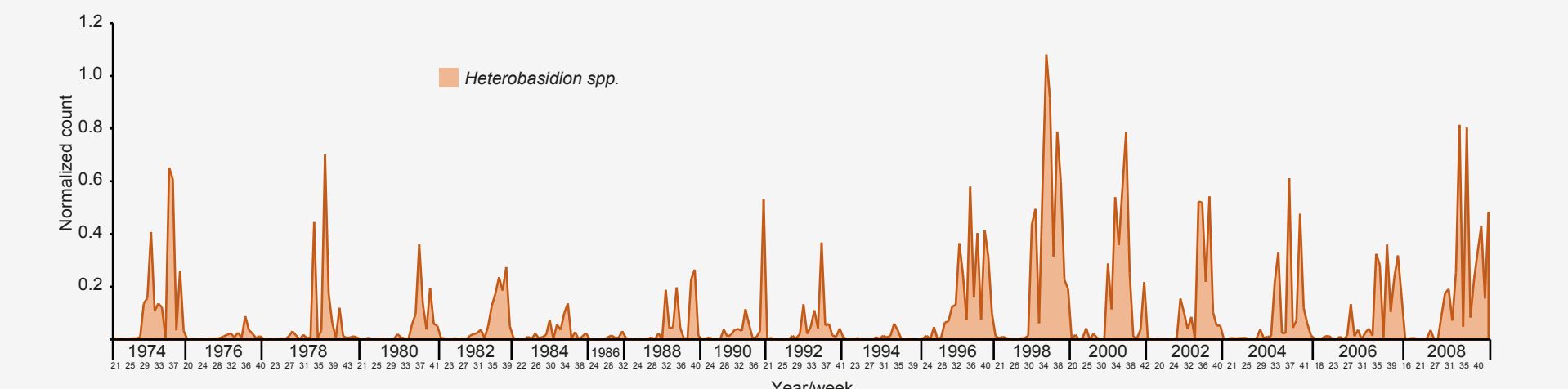


Figure. Weekly normalized relative abundance for the causative agent of root rot - *Heterobasidion spp.*

External validation

Sequence data comparison with external pollen data show that the air filters can be used to accurately track presence of organisms in air over time.

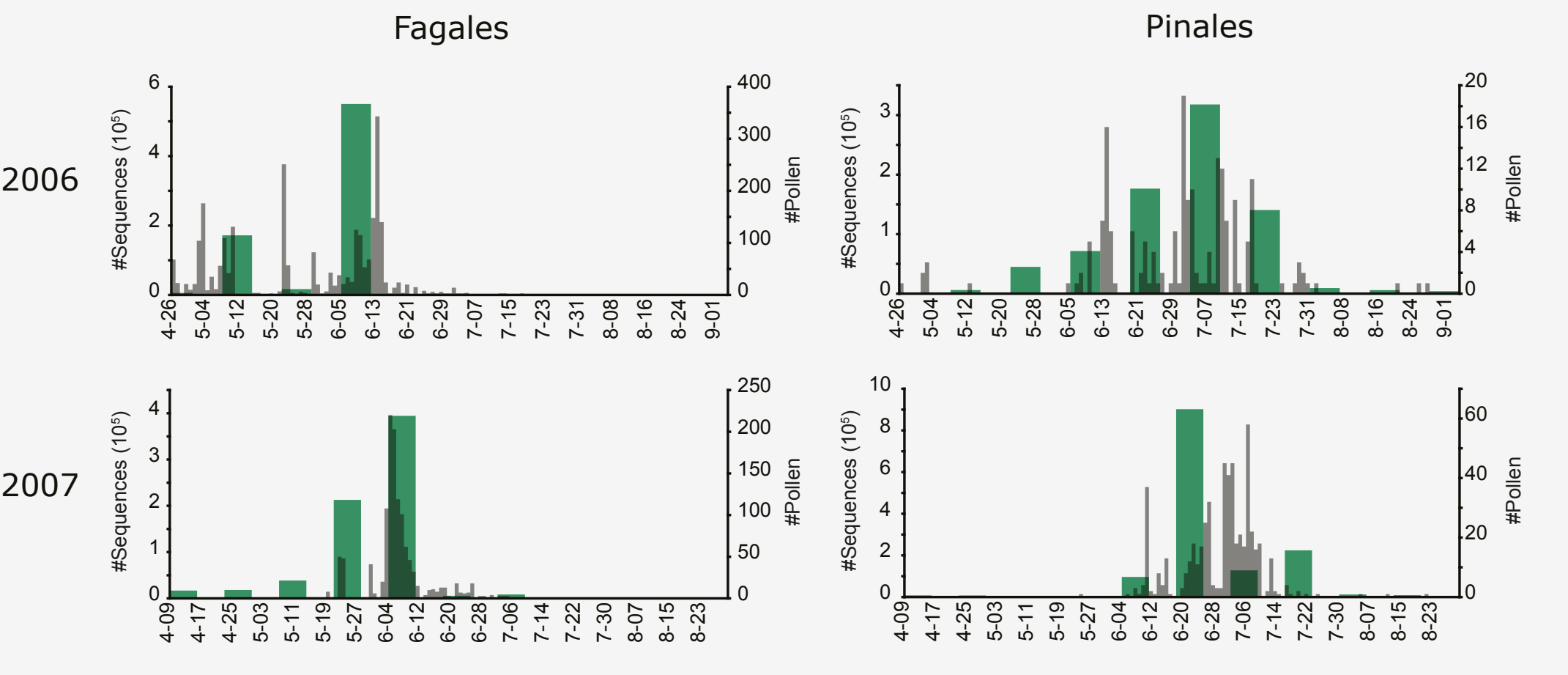


Figure. Comparison between Kiruna sequence counts (per every second week, green) and Abisko pollen counts (per day, gray) for the taxonomic orders Fagales and Pinales.

Antibiotic resistance

The archive do not only allow us to study biodiversity, but also individual genetic traits such as antibiotic resistance in the environment over time.

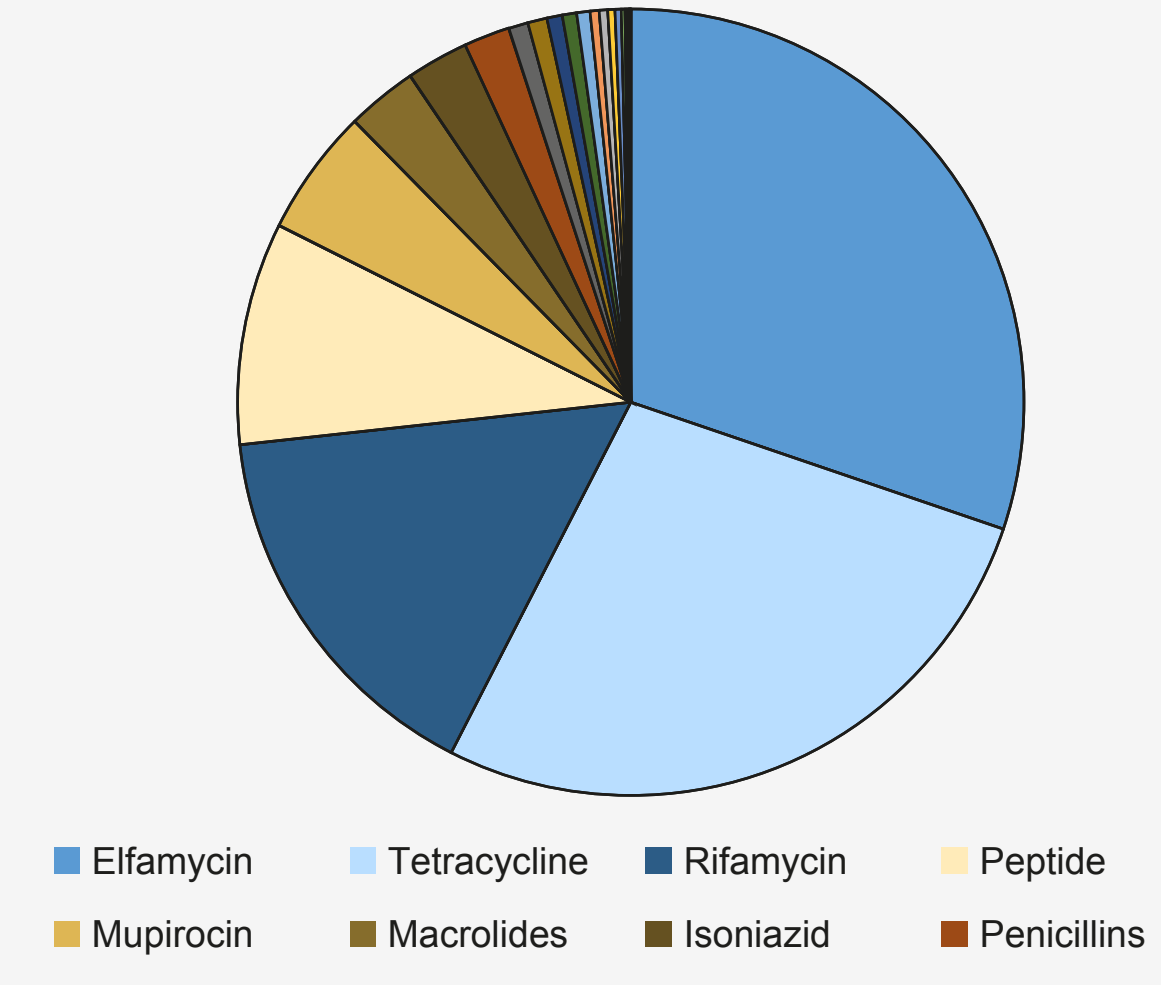


Figure. Relative abundance of DNA sequences associated with antibiotic resistance in air sampled in Gävle week 21, 2017.

Plant phenology

The archive allows us to study seasonality of pollen dispersal and possibly deduce the underlying factors that cause shifts in the timing and intensity of pollen dispersal.

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Preliminary data analysis show:

- The maximum relative abundance of pine is highly consistent between years.
- The maximum relative abundance of spruce varies considerably between years.

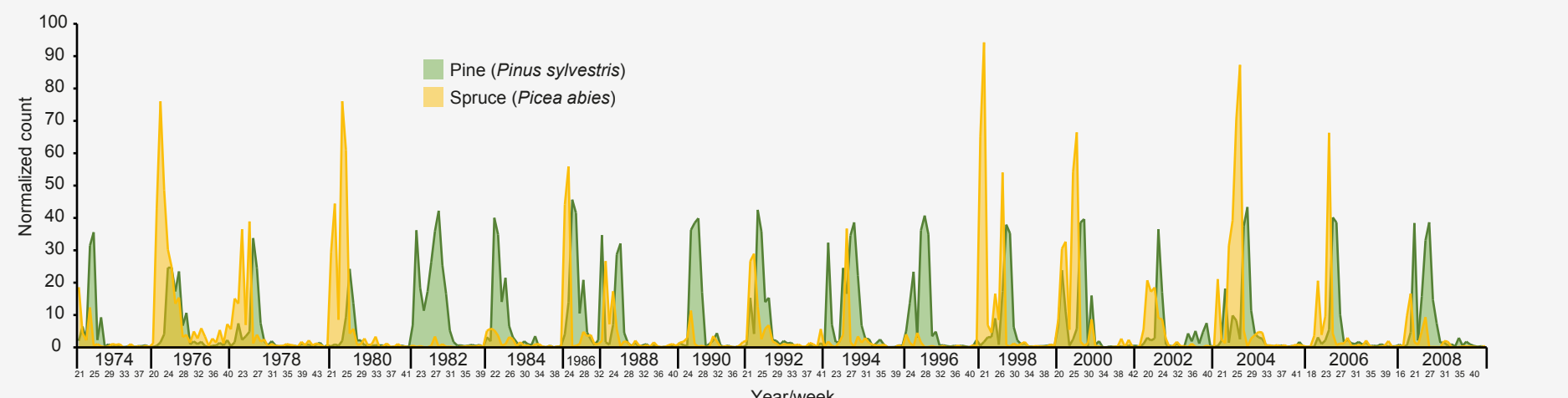


Figure. Weekly normalized relative abundance for Pine (*Pinus sylvestris*, green) and Spruce (*Picea abies*, yellow)

References
 1. Fordham, D. A. et al. Predicting and mitigating future biodiversity loss using long-term ecological proxies. *Nat Clim Change* 6, 909-916 (2016).
 2. Mihoub, J. B. et al. Setting temporal baselines for biodiversity: the limits of available monitoring data for capturing the full impact of anthropogenic pressures. *Sci Rep* 7, 41591 (2017).
 3. Kim D, Song L, Bretwieser FP, and Salzberg SL. Centrifuge: rapid and sensitive classification of metagenomic sequences. *Genome Research* 2016
 4. NCBI nt database. Bethesda (MD): National Library of Medicine (US), National Center for Biotechnology Information; 2004 - Available from: <https://www.ncbi.nlm.nih.gov/>