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Project *brief*

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eDNA rainwash sampling: A novel approach for biodiversity monitoring in forest ecosystems

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- **Environmental DNA (eDNA) metabarcoding is a minimally invasive, low-cost, and comprehensive approach for assessing and monitoring biodiversity and its loss in forest ecosystems.**
- **A pilot study on rainwash sampling at the Britz Intensive Forest Monitoring Station resulted in high taxonomic resolution and shows potential for closing the knowledge gap on the composition of invertebrate and fungi communities associated with tree crowns.**
- **We propose a more comprehensive study with the aim to develop a standardized protocol for eDNA analyses in traditional forest biodiversity monitoring.**

Background and aims

eDNA metabarcoding has become a promising tool in ecology and biodiversity management. It comprises the taxonomic identification of multiple species or species groups from DNA fragments in environmental samples, e.g., streams, soil, feces, or air, by PCR amplification and high-throughput sequencing and has been used for different taxonomic groups and biomes since 2012. Compared to traditional sampling methods and species identification, eDNA metabarcoding provides a minimally invasive, relatively quick, low-cost, and comprehensive overview of the diversity of species in a sampled ecosystem, especially when species are rare and difficult to access, such as in tree canopies.

The major aim of this pilot study was to assess the potential of eDNA metabarcoding from rainwash sampling for forest biodiversity monitoring. Specific objectives were to test the effect of overstory tree species, time of year, length of sampling period, throughfall vs. stemflow sampling, precipitation volume, and disinfecting of samplers on the insect and fungi community composition in tree canopies.

Materials and methods

We sampled precipitation on four 0.3 ha stands at the Britz Intensive Forest Monitoring Station: two European beech (*Fagus sylvatica* L.), one Scots pine (*Pinus sylvestris* L.), and one sessile oak (*Quercus petraea* (MATT.) LIEBL). The age of all stands was 50, except for the oak which was 25.

We collected rainfall with Hellmann rain gauges and collectors of the same design under the tree canopy (throughfall) in all four stands and from stemflow in two stands (beech, oak) for 28 days, resulting in a total of 19 samples from March 2024 for eDNA analyses (Fig. 1 and 2). Additional samples were taken in June and October, but results are not yet available.

Rainfall samples were filtered and directly sent to the lab for further processing. After the extraction of DNA, markers specific for invertebrates and fungi were amplified using specifically designed primers with PCR and the resulting sequences were assigned to corresponding taxa using public DNA reference databases.

Figure 1: Precipitation samplers for throughfall (left) and stemflow (right) at the Britz Intensive Forest Monitoring Station (Source: A. Michel).

Key findings

For invertebrates, 337 Operational Taxonomic Units (OTU) could be assigned to a species (30.6%), genus (14.5%), family (32.3%), order (22.0%), or at least class (0.6%). The dominant phylum was arthropods and the three most dominant classes were springtails, arachnids, and insects.

The number of OTUs was highest in European beech (245 OTUs), followed by sessile oak (174 OTUs), and Scots pine (54 OTUs), with some OTUs restricted to a specific tree species. Stemflow resulted in a higher α-diversity, i.e., species richness, than throughfall with molluscs having been exclusively detected in stemflow.
For fungi, 616 OTUs were found belonging to 6 fungal phyla, 21 classes, 66 orders, 133 families, and 203 genera. Most fungi were ascomycetes (72.0%), 18.2% were basidiomycetes, and 9.0% could not be identified at a higher level than fungi. More rare phyla, such as Mucoromycota, Zoogagomycota, and Chytridiomycota, were also found. α-diversity was similar in beech and oak but smaller in pine. The fungal community composition was similar among samples of a single tree species but differed between species, and around 2/3 of the detected OTUs were restricted to a specific tree species. The community composition was also different for throughfall and stemflow sampling.
In both analyses, filtered water volume between 330 and 1000 ml had no effect and the differences between new and uncleaned samplers were statistically significant yet small in one of the analyses and thus require further investigation. Although we expected DNA degradation as a result of the comparably long sampling time of 28 days, many species were detected after one month in March. These results will still be compared with data from June and October, which are currently being analyzed.

Conclusions

- Rainwash eDNA can produce biodiversity data with high taxonomic resolution.
- Stemflow is a valuable addition to throughfall sampling for detecting invertebrates and fungi associated with trees.
- A filtered water volume of 330 ml or possibly even less seems sufficient but this needs to be studied in more detail.
- This also applies to the effect of cleaned vs. non-cleaned samplers, between which only a small difference was found.
- Many species could already be detected as early in the season as March.
- A one-month sampling period, as is typical for traditional forest monitoring, seems feasible but DNA degradation later in the year still needs to be tested.
- We propose a larger follow-up study to confirm these results and their applicability to other forest ecosystems.

Advice for policy-makers

Next to climate change, the loss of biodiversity is one of the most pressing threats to human existence. Monitoring biological diversity is therefore absolutely crucial for the understanding of the speed and scope of global biodiversity loss.

This pilot study shows that eDNA in forest biodiversity monitoring could play an important role in understanding and preserving forest ecosystems and their biological diversity. First results from the March 2024 sampling indicate that the applied workflow is suitable for the analysis of invertebrate and fungi environmental DNA from rainwater samplers and may be used to complement traditional sampling and identification of biodiversity in forest monitoring.
Only 31% of OTUs from invertebrates and 34.1% from fungi could be identified at species-level so that OTUs will continue to serve as proxy for species until reference barcoding databases will be completed. The further development of comprehensive eDNA reference databases to accurately assess biodiversity should therefore become a top priority.
Future research should focus on refining eDNA sampling methods and include larger sample sizes in different media (e.g., tree canopies, soil, air, deadwood, tree microhabitats) with the aim to develop a transnational standardized protocol for the use of eDNA in forest biodiversity monitoring in Europe. This could be supported by the existing forest monitoring network in Europe and beyond established in 1985 as the International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forests (ICP Forests) under the UNECE Air Convention and co-ordinated by the Thünen Institute of Forest Ecosystems.

Figure 2: Precipitation samples before filtering (Source: A. Michel).

Further Information

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