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EGU General Assembly #EGU25 27 April - 2 May 2025

Ectomycorrhiza: Back to the future

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Introduction

High throughput sequencing allows us to have deeper insights into the occurrence and diversity of species via **metabarcoding**. This includes under-explored mycorrhizal fungi, with the advantage of **not being dependent on fruiting bodies.** Our project “Back to the future” analyses archived forest soil samples from the past 32 years in order to capture diversity of ectomycorrhizal fungi. The dataset comprises samples from areas ranging from meadow forests of Pannonian regions to montane forests of the inner Alps. The **historical samples** can be used to record changes in the community composition over time, with particular consideration of **climate change** and **loss of species**. To this end a highly accurate affiliation of OTUs is of utmost importance. All OTUs classified as ECM species underwent manual curation for improved species identification. Initial analyses detected >7000 fungal species, including >800 species of ectomycorrhizal (ECM) fungi.

Material and Methods

- 28 forest sites across Austria (Fig.2)
- Historical and recent samples from the 1990ies until today
- DNA extraction, amplification of ITS2 region (Tedersoo et al., 2014), Illumina MiSeq sequencing
- OTU clustering within 3% sequence variation
- Assignment of species with UNITE 10.0 - All Eukaryotes Database (Abarenkov et al., 2024)
- Assignment of Ecological guilds at the genus level (Gorfer et al., 2020)

Figure 1: Zöbelboden, 2024

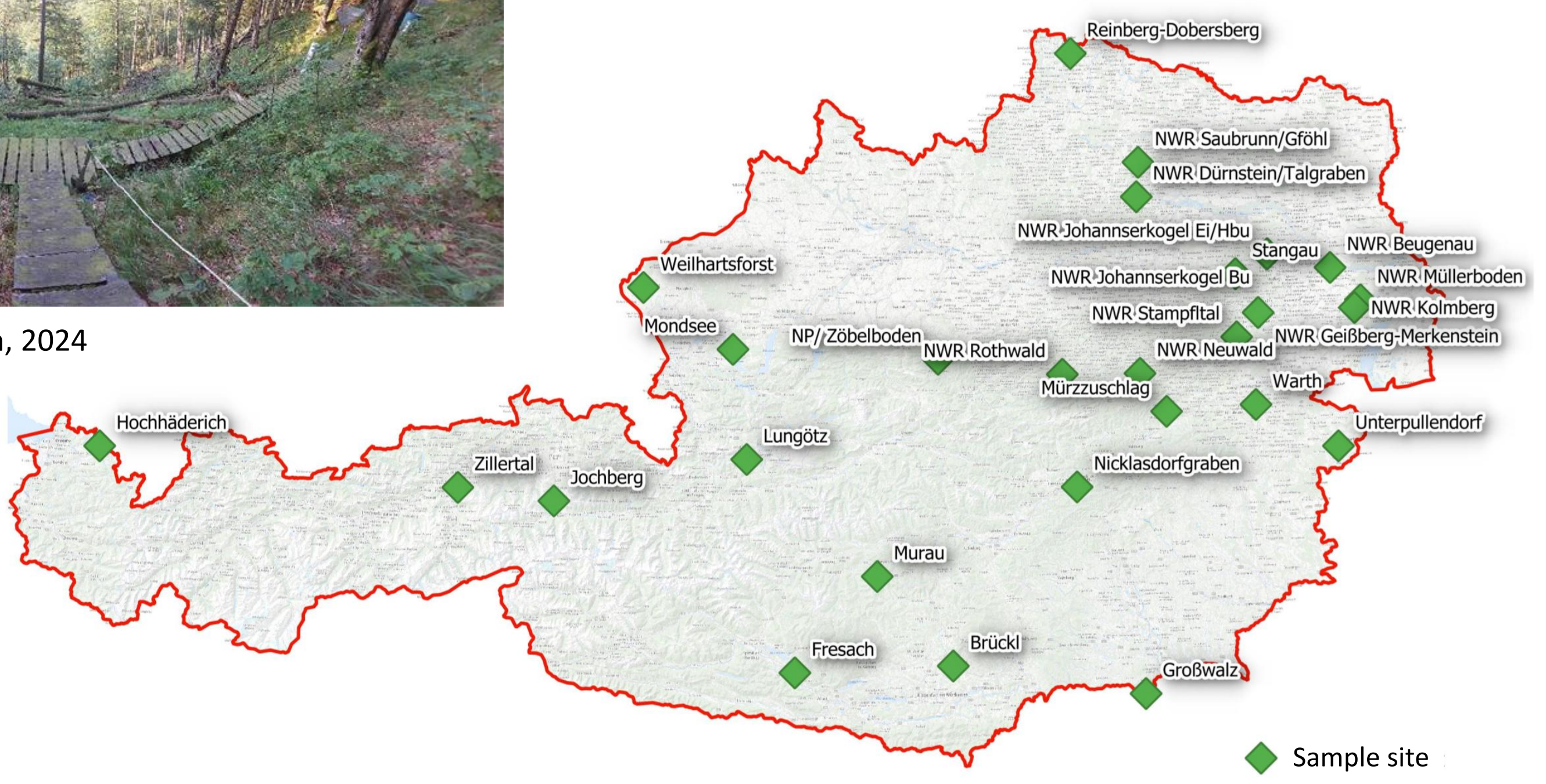

Figure 2: Location of sample sites in Austria

Figure 3: Sampling in Zöbelboden, 2024

Inquired Databases

- NCBI
- BOLD
- PlutoF
- Gbif

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Species identification

Standard pipeline:

- 16,245 different OTUs were defined
- 9,143 identified as fungi
- 1,092 identified as ECM genera
- 614 identified as ECM species

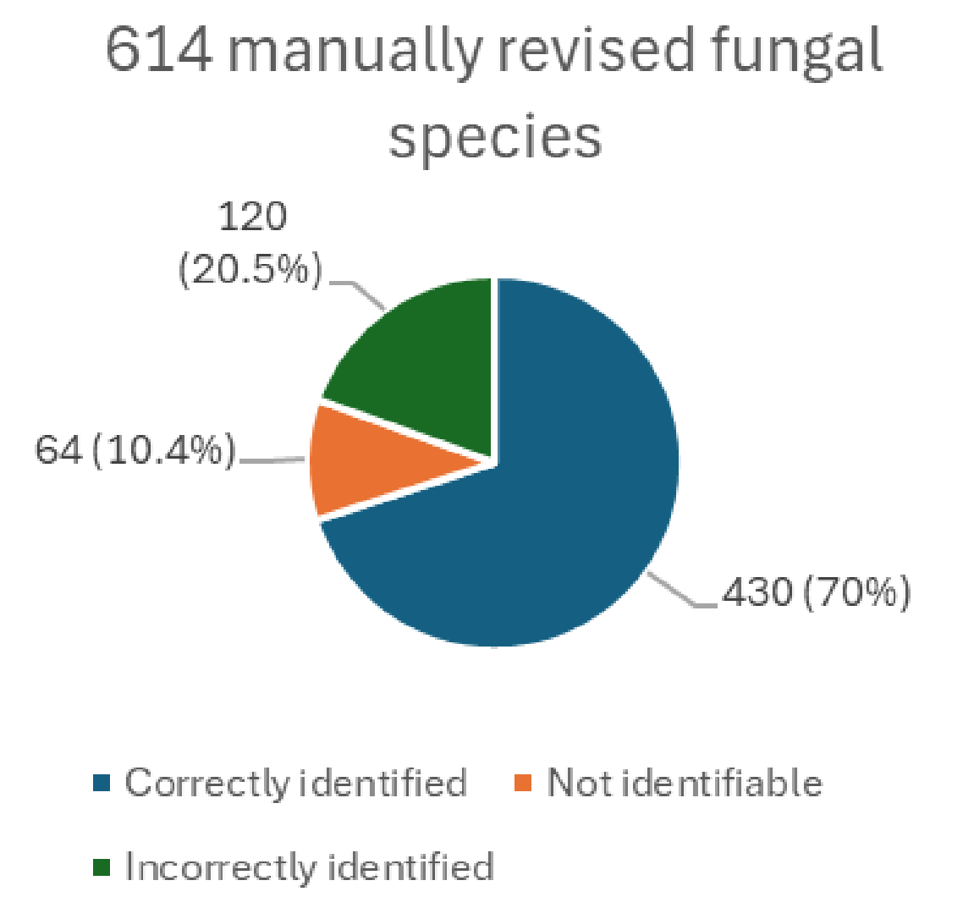

Figure 4: Manually revised ECM species results

Manual revison of ECM species:

- 430 (70%) retain their species name
- 120 (19.5%) corrected
- 64 (10.4%) couldn’t be distinguished
- a single sequence had a deviating genus

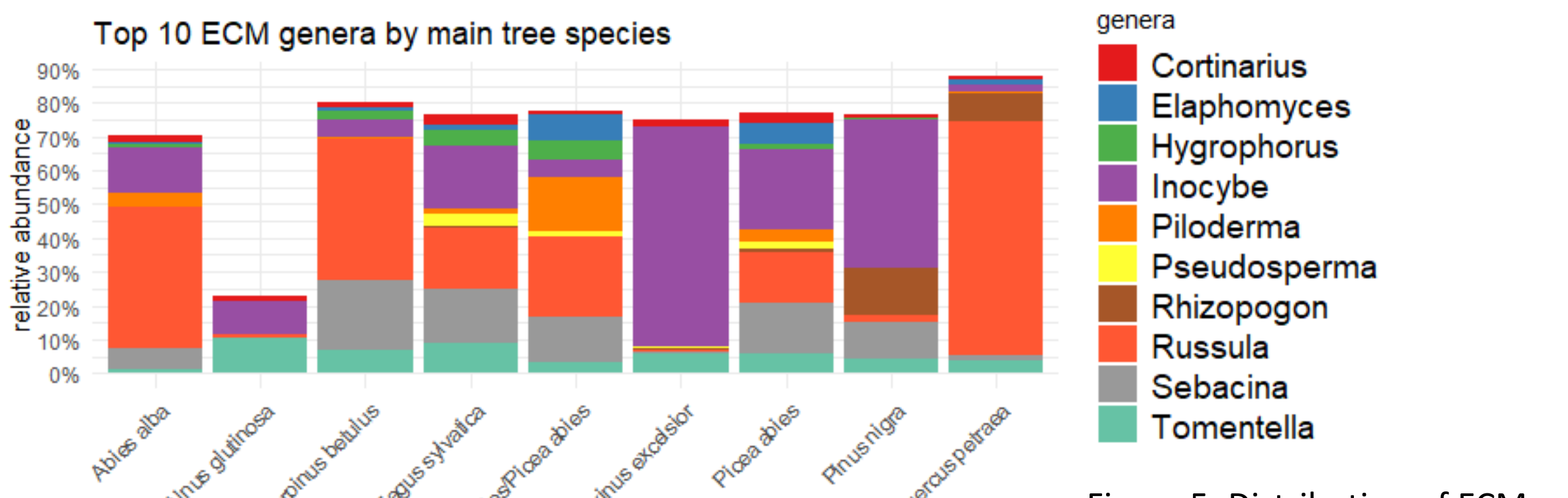

Figure 5: Distribution of ECM genera by main tree species

Detected rare fungi by metabarcoding

Figure 6

Figure 7

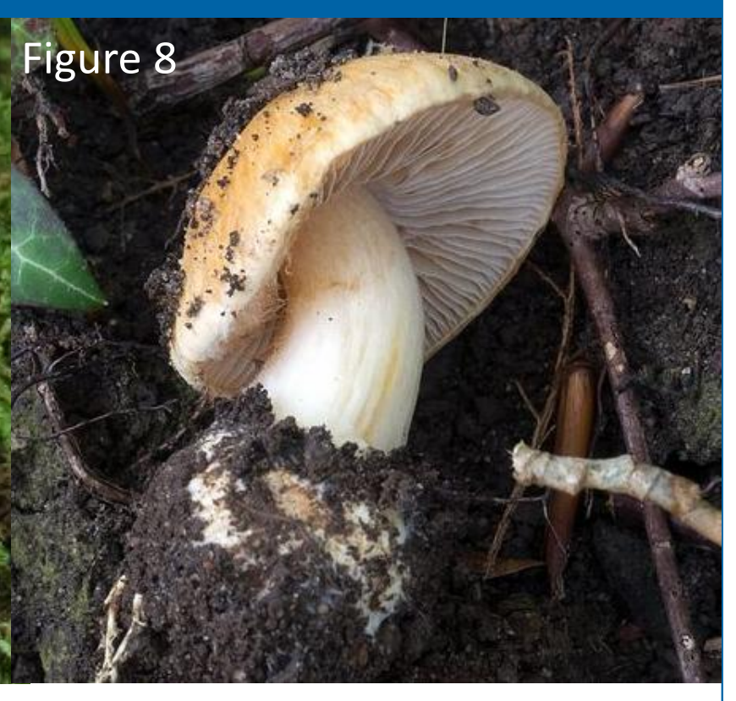

Figure 8

From left to right: *Hydnocystis piligera, Hydnum mulsicolor, Phlegmacium cruentipellis*

Conclusion and outlook

- Standard pipeline identification has a low accuracy (up to 30% error) on a species level
- Standard pipeline identification has a near 100% accuracy on a genus level (0.16% error)

- Sampling and genotyping of fruiting bodies of rare fungi
- Further monitoring and changes over time
- Identifying environmental and site specific impacts
- Targeted approach for detecting endangered species of ECM fungi

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Figures:

1: Dukic, Ika 2024
2: BFW, 2024
3: Dukic, Ika 2024
4: Ploderer, Matthäus 2025
5: Hasenzagl, Magdalena 2025
6: Hydnocystis piligera: https://www.gbif.org/occurrence/5007270719 (04.2025)
7: Hydnum mulsicolor: https://www.gbif.org/occurrence/4054908452 (04.2025)
8: Phlegmacium cruentipellis: https://www.gbif.org/occurrence/2238515670 (04.2025)