Metabarcoding of Quercus robur mycobiomes from the "Meteorite Morasko" Nature Reserve as a source of referential data for Carpinion betuli forests

Maksymilian Chmielewski [12], Mikołaj Charchuta[12], Władysław Polcyn [1]
Adam Mickiewicz University, [1] Department of Plant Physiology, [2] Bioinformatics Section of Natural Sciences Club
makchm@st.amu.edu.pl

Reserve areas protect biological communities important for biodiversity management as the referential data, allowing us to qualify other places as worthy of conservation. We have analysed soil DNA samples from one such place - "Meteorite Morasko" Natural Reserve. It is a place known for its abiotics wonders, but given the presence of old Carpinion betuli forests we had a reason to think it will be rich in interesting fungi too.

The fungal ITS1 region was PCR-amplified and then sequenced with NGS technology, using the Ion Torrent platform. H**aving access to such high-level techniques, vulnerable to systemic errors, we remain vigilant for random data.** Such errors can come from sampling, sequencing and analysis or simply the inherent chaotic nature of biological data. **Therefore there exists a need for robust data validation, which was performed herein (Fig. 1 & 2) using tools of computational statistics.**

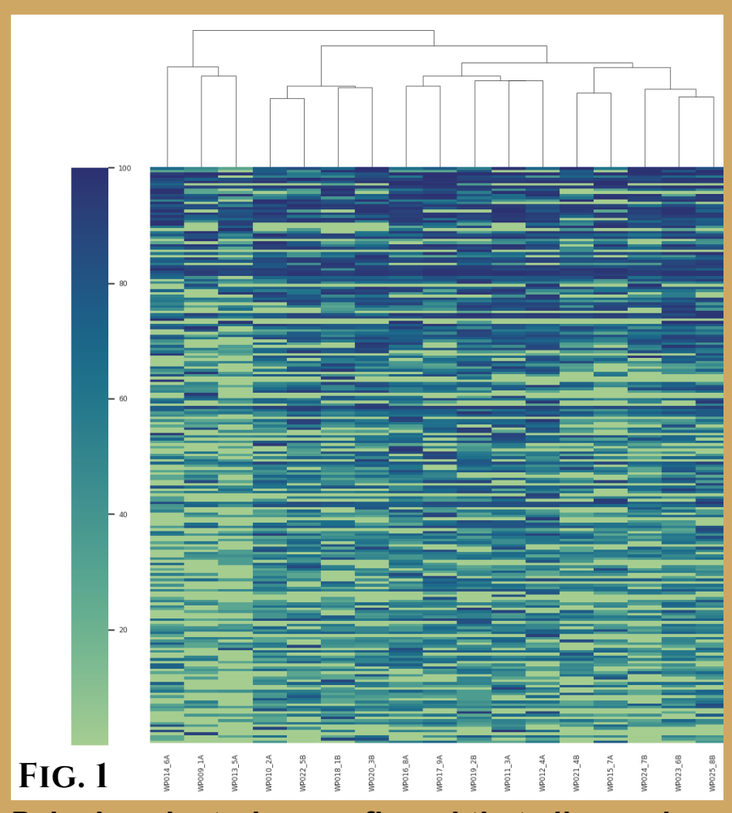

Fig. 1

Pairwise clustering confirmed that all samples taken (17 trees) constitute a single cluster.

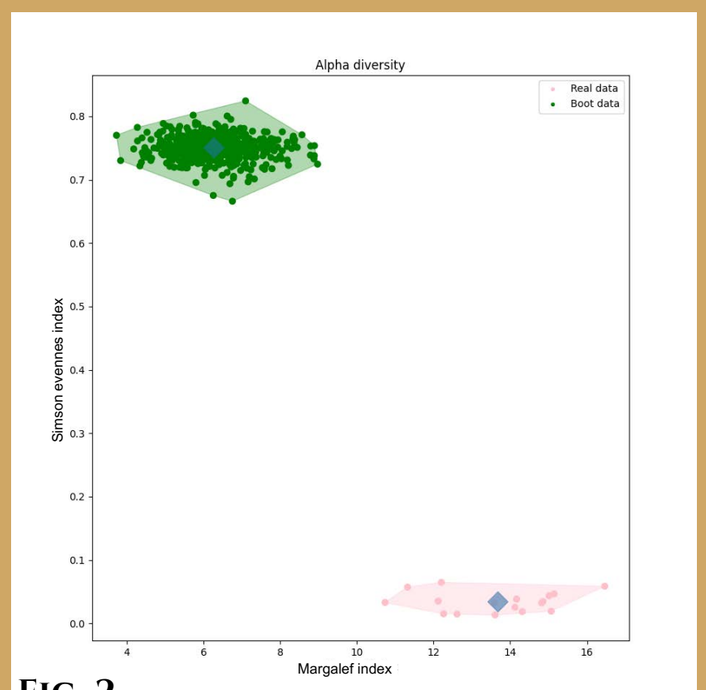

Fig. 2

K-means clustering and the bootstrap method used as a validation procedure to check if the obtained data (pink) do not overlap in one cluster with a group of random data (green).

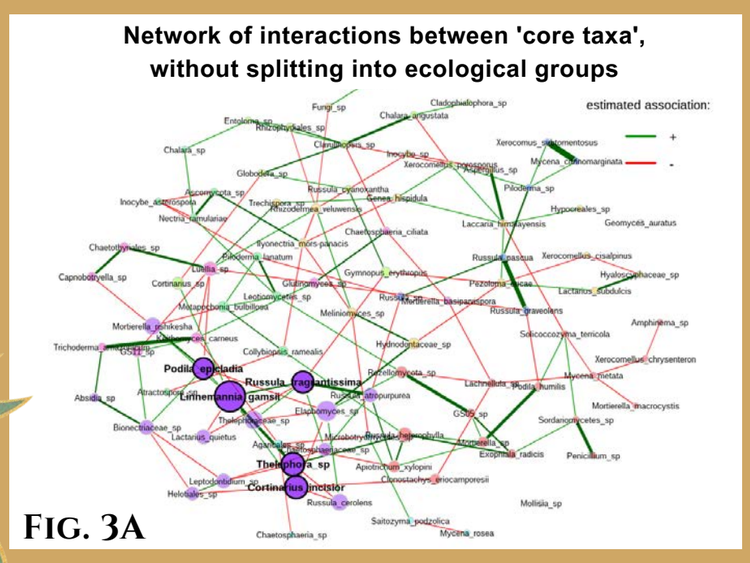

Fig. 3A

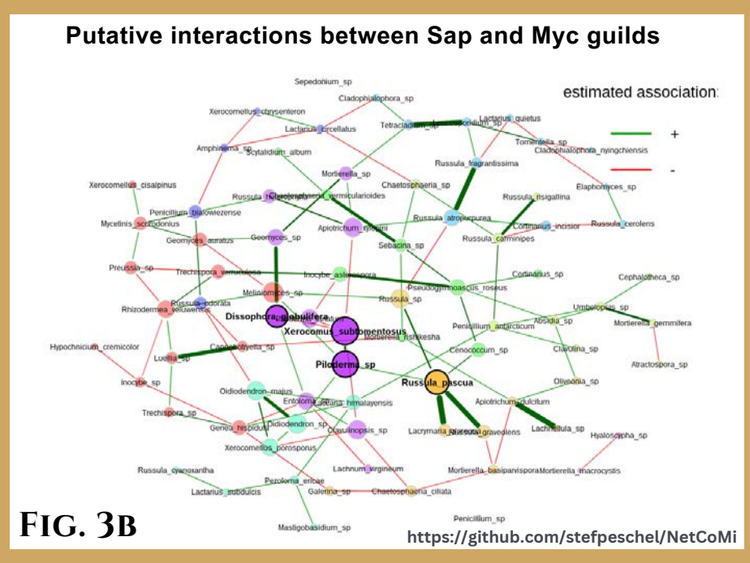

Fig. 3B

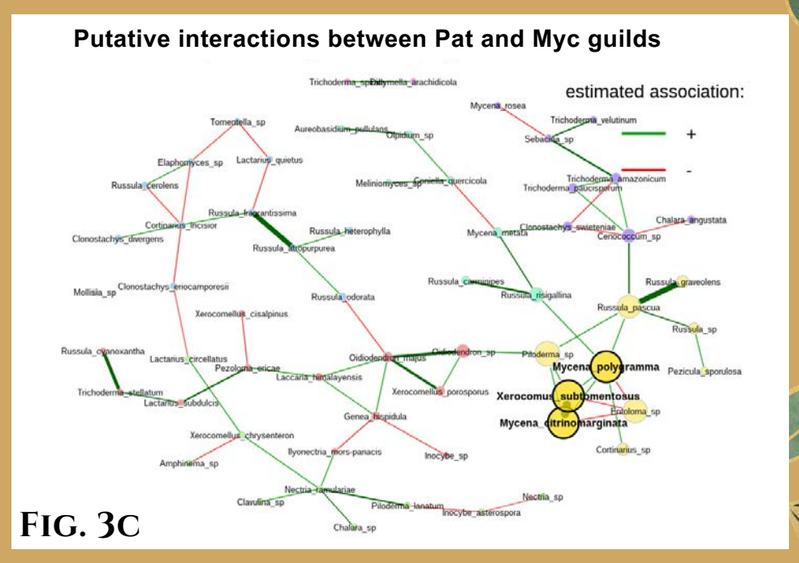

Fig. 3C

Fig. 3. The FUNGuild tool was used to divide the 820 relevant taxa into putative plant-oriented ecological groups, on which only 4 (Sap - saprohytes, Myc - mycorrhizas, Pat - pathogens, Nop - non plant-oriented) were populated by taxa passing filtering criteria based on occurence (>=100 sequences found). This left 217 species covering over 99% of the total number of hits. Then the putative interactions between 217 'core taxa' (A) and cross-guild interactions (B &C) were visualized by using the NetCoMi R package. Only species with >150 hits were shown for clarity.

Fig. 4. The Krona visualisation of taxonomy and ecology grouping, (A) taxonomy chart of 217 taxa after filtering, (B) the same species ascribed to plant-oriented ecological groups.

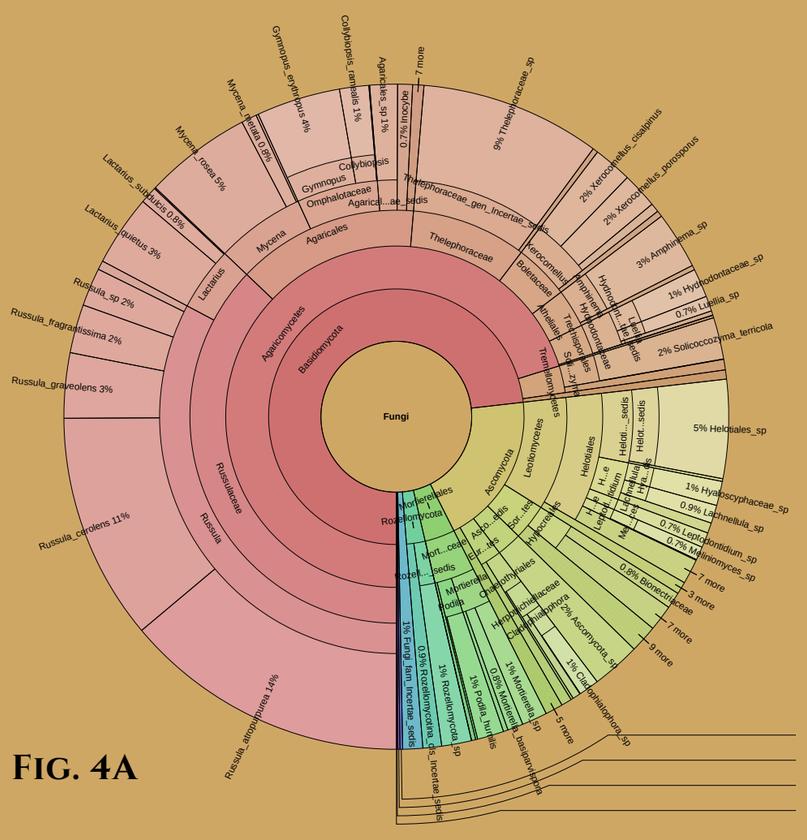

Fig. 4A

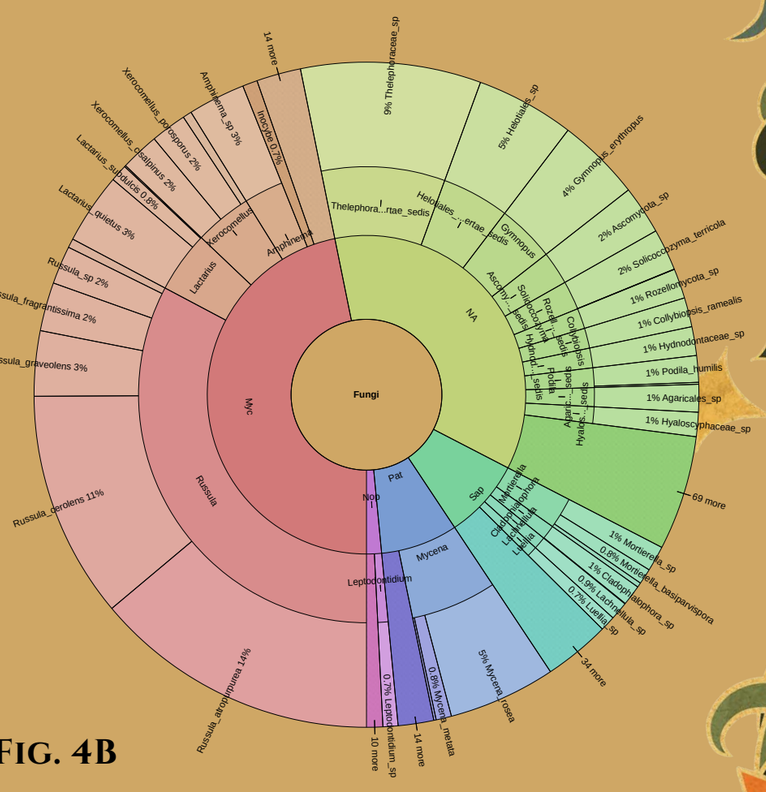

Fig. 4B

Issues

The employed software definetly can benefit from more data. In fact, supplying the pipeline with enough data to satisfy its needs proved to be the main computational challenge.

Conclusions

THE QUALITY OF THE DATA OBTAINED WITH THE PRESENTED VALIDATION TOOLS ALLOWS US TO RECOMMEND IT FOR ANALYSES OF SIMILAR ENVIRONMENTAL DATASETS.

All data and scripts are available in our github repo, available under this QR code:

Acknowledgements

- **The ID-UB funding innitiative of AMU for two students grants in molecular ecology**
- **The Dean of AMU Faculty of Biology and the Natural Sciences Club for supporting our research**
- **Society for the Protection of Underground Networks (SPUN) for their efforts in mapping out mycorrhizae**