

Description of the project: „Mycorrhizal communities associated with monumental trees in the Bieszczady part of Polish Carpathian primal forests”.

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We are one of 31 teams that participated in the 2023 global scientific project SPUN (Society for the Protection of Underground Networks)¹. We have led expeditions to the south-eastern part of the buffer zone of the Bieszczady National Park (BdPN) to provide knowledge about the species diversity of the mushroom communities living there. For this purpose, we sampled soil DNA in the rhizosphere (root zone) of monumental beeches and firs. These trees have reached the size of natural monuments, so it can be expected that both they and the microorganisms found in the soil surrounding their smallest roots co-exist in a density that can be considered the so-called hot spots of biodiversity in the mountain forests of this region.

The main idea of SPUN is to create a global map of hot spots of mycorrhizal species, taking samples in the places least disturbed by humans. This is to initiate global conservation efforts for mycorrhizal ecosystems. These symbiotic fungi are extremely important for maintaining the condition of plants in terrestrial ecosystems, as they interact with them in deep nutritional, anti-stress, and even phytosanitary interactions². They are also responsible for retaining organic carbon in the soil, which in a world without mycorrhizae would be released into the atmosphere as a greenhouse gas in an amount equivalent to approximately 36% of current annual CO₂ emissions from fossil fuels³.

In addition to mycorrhizae, our team also identified other fungal ecological groups living in the rhizosphere of beeches and firs, i.e., other symbiotrophic, pathogenic, and saprophytic species. Each of these species is an integral part of the soil ecosystem, as it interacts with the plant part of forests in complex, and mostly not yet recognised, interactions. Artists from the University of the Arts in Poznań, i.e. Prof. Joanna Hoffmann-Dietrich, Jadwiga Subczyńska and Maria Subczyńska, are also involved in the project. They took part in our expeditions, and their artistic sensitivity will help all of us feel part of the world of Nature. Our communities have been cooperating with each other for years in an artistic, scientific and educational project „Rhizosphere: The Big Network of Small Worlds”. On the website of this project you can find information about the joint expeditions of SPUN ⁴.

DNA samples were collected during two visits to the Bieszczady Mountains, in May and October 2023. We explored 10 places, finding 35 beeches and 35 firs the size of natural monuments, indicated to us by Piotr Klub, a forester and activist of the Natural Heritage Foundation. Piotr is the author of the map of the buffer zone of the BdPN, containing the locations of 3675 trees of monumental size, of which we visited only some of them.

In previous years, these trees were exposed to destruction as a result of predatory forest management that did not respect their natural value. Fortunately, shortly after the end of our research, there was a great political change in Poland, thanks to which a ban on cutting down such trees in this region was introduced. We are proud to have joined the long-term effort of activists and scientists, which has led to a historic change in the approach to the protection of the earth's natural heritage in Poland. Now we expect that the places we have studied will be included in the Bieszczady National Park. Piotr Klub

¹ <https://www.spun.earth/expeditions/underground-explorers>

² <https://www.autoportret.pl/artykuly/lepiej-razem-niz-osobno-symbiotyczna-wyprawa-do-lasu/>

³ <https://www.sciencedirect.com/science/article/pii/S0960982223001677>

⁴ <https://artscience-node.com/spun-mycorrhizal-wealth-of-carpathians/>

is the author of a comprehensive study indicating a list of places that should be covered by the highest forms of protection in Poland⁵.

The loss of biodiversity is considered one of the greatest threats facing humanity today. For this reason, scientific organisations are calling for fungi to be recognised as a target for the protection of the Earth's biodiversity. The world of fungi should be protected on an equal footing with animals and plants as the "third F", namely "flora, fauna & funga". Our record of rich fungal biodiversity can be used as reference data for other beech and fir mountain forests. It can be used to assess degradation processes, e.g., those related to forest management pressures or climate change, causing an increase in average annual temperatures and soil drainage.

Natural valorization was carried out using methods other than traditional ones, which could identify mainly species of macrofungi, i.e. those that were recognized by a specialist-mycologist by their characteristic macroscopic morphological features, or as a result of laborious microscopic or biochemical treatment. We have reached for the most modern analytical tools, i.e. devices for fast, large-scale, and precise recognition of DNA sequences from soil samples, the so-called new generation sequencers.

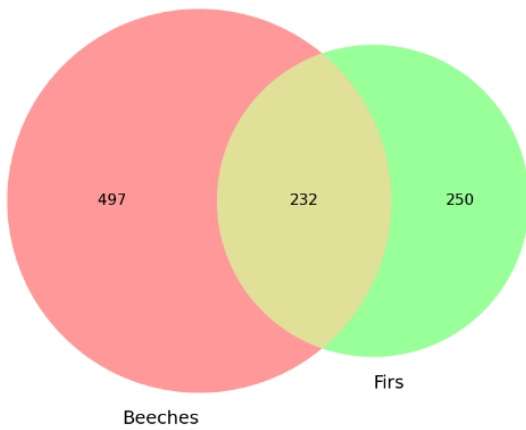
These state-of-the-art techniques require scientific teams not only to have ecological knowledge, but also advanced programming skills and knowledge of computational statistics. This task was taken on by bioinformatics students, Maks Chmielewski and Mikołaj Charchuta, who not only assigned the identified DNA sequences to fungal species, but above all proposed innovative solutions in the field called "Big Data Approach". These are ways to interpret large molecular data sets by processing, analysing, and visualising them. They make it possible to extract and organize data from "big data" sets, often with an uncertain structure, also according to ecological hypotheses that we have tried to formulate in heated and long-lasting discussions.

You can see the results of these investigations on the visualizations of the summaries shown below. They are supported by solid statistical premises, which allow us to distinguish groups of fungal species that most often accompany each other in the places in the Bieszczady Mountains, and thus co-create mountain forest habitats together with trees. We propose divisions into group categories not only according to taxonomic affiliation, but also by recognizing "clans" with established trophic habits that co-occur in the microhabitats of individual firs or beeches. For this purpose, we use bioinformatics tools to analyse network relationships.

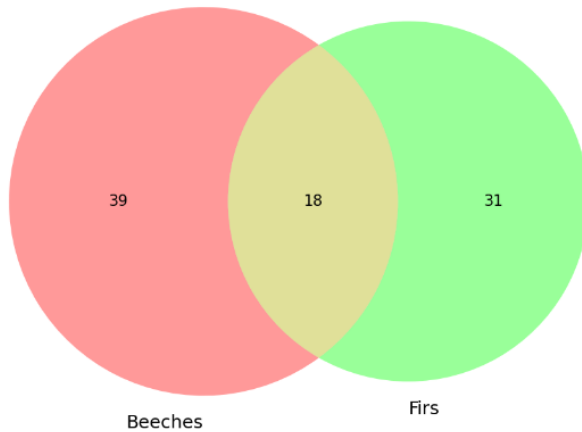
In conducting these analyses, we relied on reference databases that assign unique DNA sequences to fungal species and their lifestyles, previously identified by other types of techniques – morphological, microscopic and biochemical. Such databases are growing every year, so our methodology, called metagenomics, is becoming more and more efficient in rapidly recognising the composition of soil ecosystems, not only for fungal species but also for bacterial and microscopic eukaryotes. In the process, large numbers of unique DNA signatures are being discovered, belonging to known taxonomic groups but so far not assigned to any species, because there is a lack of data describing the characteristics of these organisms.

⁵ <https://przyrodnicze.org/25-nowych-parkow-narodowych-do-utworzenia-w-polsce/>

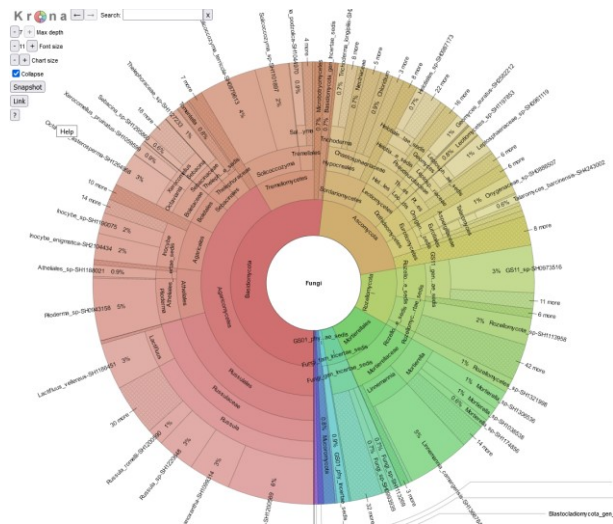
Number of all guilds species in Beeches and in Firs



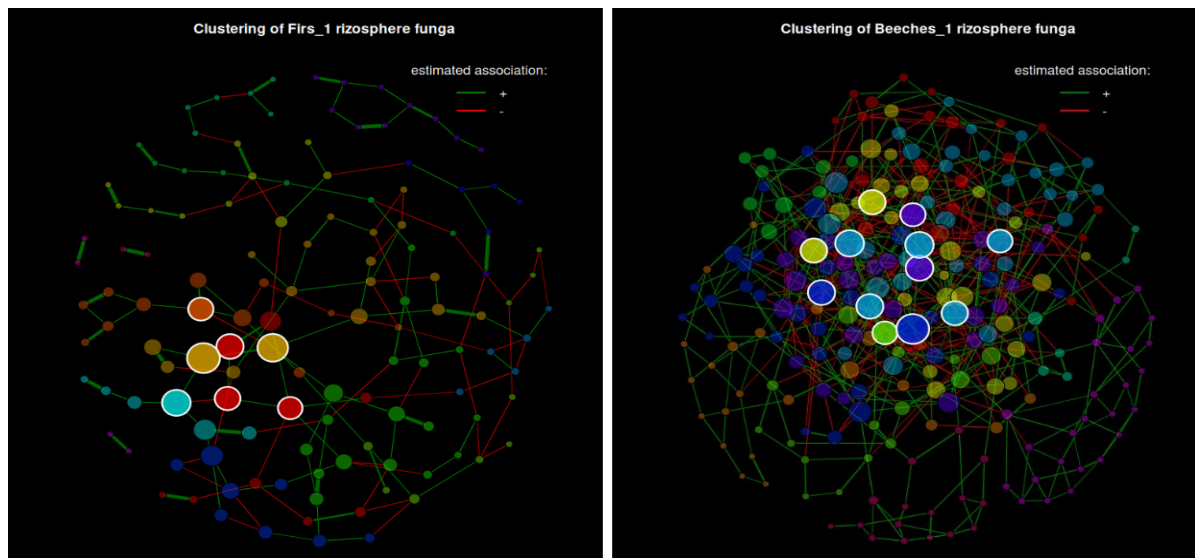
Number of Myc species in Beeches and in Firs



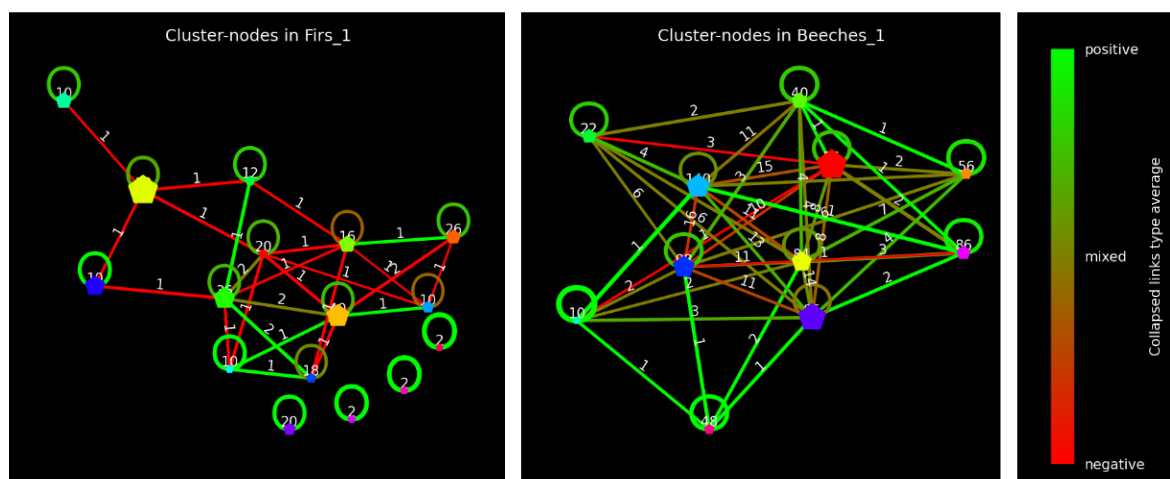
Left: identification of differences in the species composition of soil fungi accompanying monumental beeches or firs (Venn diagram). The numbers indicate fungal species specific to or common to both tree species.
 Right: Venn diagram showing the differences and similarities in the sets of mycorrhizal fungi associated with beech or fir trees.



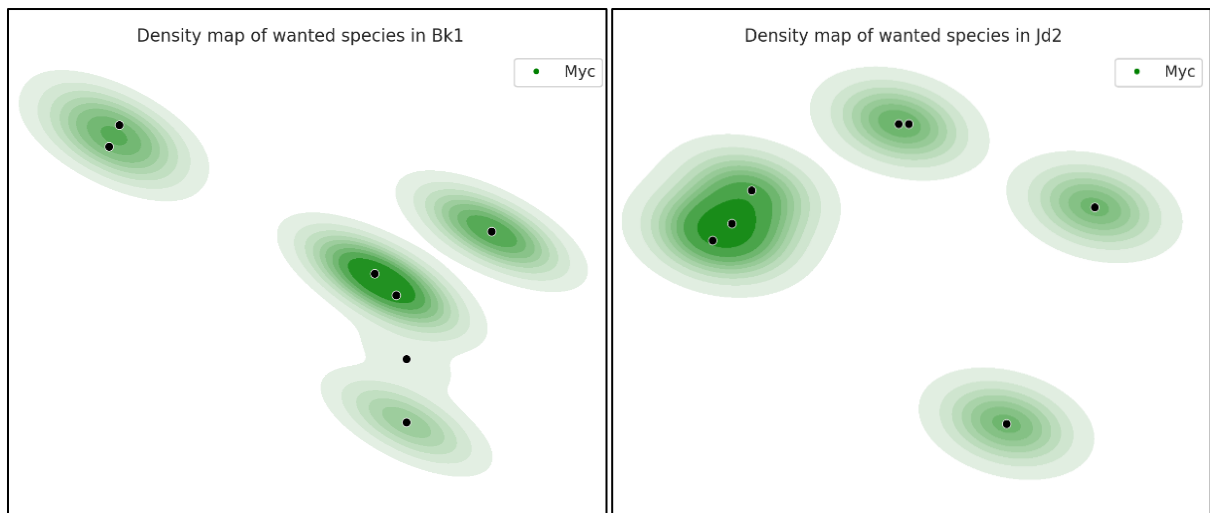
An interactive tool (Krona) to inspect the taxonomic composition and ecological functions of the species that make up the mycobiomes of the rhizosphere of the studied trees.



Graphs of fungal networks, showing differences in mycobiome structures (fungal communities) for beech and fir habitats. The green lines indicate the co-occurrence of species with a pattern repeated in the samples from the 35 trees tested for the species. The red lines indicate that the species are mutually exclusive. Circles of the same color are used to mark the so-called clusters, i.e. groups of species forming an internally cooperating "clan". A bioinformatics tool was used – NetCoMi (Network Construction and Comparison for Microbiome Data) with the use of the similarity search method (SPIEC-EASI).



A simplified picture of the network showing the relationships only between individual groups of species, i.e. the network of occurrence of "clans". Green lines – co-occurrence, red – mutual exclusion of clusters. The size of the symbol (pentagon) indicates whether the size of the "clan" (the number of species and their representatives) is larger or smaller in relation to the others.



Exemplary maps predicting the approximate density of mycorrhizal fungi around monumental beeches or firs that form two selected forest habitats. The computational technique "2D Gaussian kernel density estimation" was used.