

In the last 50 years, humans have altered ecosystems faster and more intensively than in any comparable period in their history. This has resulted in a significant and largely irreversible loss of the diversity of life on Earth. Biodiversity has a positive impact on many ecosystem functions. Forest ecosystems are the most important for biodiversity - they are home to about 80% of terrestrial species and cover only about a third of the Earth's land area.

Trees play a key role in their structure and function as the dominant primary producers. Microbial consortia, including fungi, bacteria and archaea, inhabit a variety of forest habitats. The availability of nutrients affect the number of micro-organisms and the composition of their communities. In natural forests, the amount of dead wood may be greater than that of living trees. In our study site - the Białowieża Forest - one of the best preserved European temperate forest ecosystems, dead wood represents about 30% of the biomass of the stands. It seems that this biomass is important because it is involved in the carbon cycle, but it is an important habitat for a wide variety of microorganisms.

The planned research is based on the hypothesis that during the decomposition process of dead wood, which can take several tens of years depending on the environmental conditions, there is a significant change in the composition of the wood-degrading microbiome over time, from the dominance of saprotrophic to mycorrhizal species.

Since 1964, the research team from the Białowieża Geobotanical Station of the University of Warsaw has been carrying out ecological monitoring of dying and falling trees on a 1 ha permanent research plot in the strictly protected area of the BPN. This monitoring allows the dating of dead wood lying on the forest floor and creates a unique opportunity to analyse the complex processes related to wood degradation, including the biodiversity of organisms involved in this phenomenon as a function of time. We expect that the study of microbial consortia occupying different ecological niches and the determination of their functioning, activity and interrelationships through multidimensional analyses will allow us to better understand the complex relationships and changes taking place in forest ecosystems.

The proposed research will involve the analysis of samples of decaying wood of Norway spruce *Picea abies* (the most common deadwood species in the research plot) stems in the period from 1964 to the present (at approximately 10-year intervals since the stems fell on the litter), collected from relevant sites in a research area in the BSG UW of BPN. In this plot, all logs were identified, measured and mapped once a decade, allowing us to accurately determine the time of their fall. In addition to analysing temporal changes in the composition of the consortia of microorganisms responsible for decomposing wood (metagenomics), a central point of the proposed project would be to determine potential differences in the metabolism and enzymatic activity of these consortia over time. To assess these changes, an RNA-Seq approach will be used to qualitatively and quantitatively analyse the metatranscriptomes of such microbial communities and determine the expression of metabolically active genes. This step will be accompanied by Biolog EcoPlates analysis, which allows for community-level physiological profiling (CLPP), effectively distinguishing spatial and temporal changes in the metabolism of microbial consortia. Finally, metaproteomic analysis will be used to assess the presence of wood-degrading enzymes used by these microbial communities. At each time point, the question of changes in the chemical composition and structure of wood will be addressed.

This is the first project to learn about the composition and activity of the microbiome of decaying spruce logs in the Białowieża National Park, which is used as a reference in forest ecology, plant biology and many other biological sciences research. The expected results will allow us to present the dynamics of the microbiome and its metabolic changes in dead spruce wood over time (decades). The project will broaden our knowledge about the functioning of primary forest ecosystems and answer numerous questions about how wood is degraded in field conditions. As a result, it will improve our understanding of the carbon cycle in forest ecosystems.