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Conference Abstract

Soil microbiome diversity in selected broadleaf forests of Belasitsa Mountain: A High-Throughput Sequencing approach

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Abstract

Soil is the most species-rich habitat on Earth (Anthony et al. 2023). Soil biota play a key role in providing a wide range of ecosystem services essential for the sustainable functioning of natural ecosystems. Recent rapid advancements in molecular techniques have significantly improved our ability to study soil biodiversity. High-throughput sequencing (HTS) has greatly enhanced targeted eDNA analysis, enabling comprehensive assessments and long-term monitoring of belowground diversity and community composition.

In this study, we investigated the diversity and community structure of the soil microbiome (fungi and bacteria) along an altitudinal gradient in non-managed forests dominated by two economically and ecologically significant species – sweet chestnut (*Castanea sativa*) and European beech (*Fagus sylvatica*) in Belasitsa Mountain, Bulgaria. As part of the Bulgarian Long-Term Ecological Research network, Belasitsa Mountain provides a valuable framework for studying the long-term forest dynamics related to global changes. The study area is characterised by steep terrain, predominantly northern exposures, and an altitudinal gradient ranging from 400 to 1500 m a.s.l. The region provides unique

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environmental conditions to monitor biodiversity changes in response to complex spatial heterogeneity influenced by tree species composition, wide altitude range, and specific climate conditions. These forest ecosystems represent ecologically significant and unique habitats, with *C. sativa* populations forming the largest chestnut stands in Bulgaria and *F. sylvatica* occurring near its southernmost distribution limit. In Belasitsa Mountain, sweet chestnut forests cover approximately 20% of the total forested area at elevations up to 1000 m, whereas European beech forests predominantly occupy higher elevations. Given the growing threats to *C. sativa* forests - such as fungal pathogens (*Cryphonectria parasitica*), climate change-induced droughts, and unsustainable forest management (Zlatanov et al. 2015) – studying soil microbiome dynamics in these forests is crucial for planning conservation and management strategies.

A total of 24 composite soil samples were collected from 12 permanent sampling plots during two sampling periods (October 2023 and June 2024). Each sample consisted of 10 sub-samples taken from a depth of 0–5 cm. Soil samples from *C. sativa*-dominated forests were collected at elevations ranging from 455 to 853 m, while those from *F. sylvatica* forests were obtained from higher elevations (1080–1434 m), mainly within the Kongura Reserve. Microbial DNA was extracted from the samples, and high-throughput sequencing was applied to target bacterial 16S rRNA (regions bV18-A) and fungal ITS2 (region fITS2-C). Additionally, comprehensive soil physico-chemical analyses were conducted, including assessments of soil texture, pH, organic carbon content, nutrient concentrations (nitrogen, phosphorus, potassium, calcium, magnesium), and heavy metal concentrations (Zn, Cu, Cd, Pb).

Taxonomical profiles for both bacteria and fungi showed no significant differentiation at the phylum level. Actinobacteriota was the most abundant bacterial phylum (mean relative abundance 54%), followed by Proteobacteria (31%), and Planctomycetota (6%). Several core bacterial genera, including *Bradyrhizobium, Acidothermus, Nocardioides, Conexibacter, Mycobacterium,* and *Solirubrobacter,* dominated the bacterial communities. Notably, a substantial proportion of bacterial sequences remained unassigned at the genus level, highlighting the need for further exploration and characterisation of soil microbiota. Fungal communities were dominated by *Basidiomycota* (mean 64%), followed by *Ascomycota* (32%), with *Mucoromycota* and *Mortierellomycota* present in lower abundances. Mycorrhizal fungi, including *Tomentella, Russula, Sebacina, Inocybe, Piloderma,* and *Cortinarius,* were the most abundant genera, forming core fungal communities in both forest types.

Alpha diversity indices showed no significant differences in fungal diversity between the two tree species. In contrast, bacterial alpha diversity varied significantly, with *F. sylvatica* forests exhibiting higher bacterial diversity than *C. sativa* forests. Beta diversity analysis identified tree species as a key factor shaping both bacterial and fungal community composition, whereas seasonal variation had no significant effect.

Our findings provide the first data on the diversity and community composition of soil microbiomes in both forest types, highlighting the significant effect of tree species on microbial assemblages. By integrating advanced molecular techniques with

comprehensive soil analyses, eDNA studies will establish a framework for long-term microbiome monitoring, enhancing our understanding of changes in microbial diversity. Understanding these patterns is crucial for long-term ecosystem monitoring and evaluating microbial responses to environmental changes.

Keywords

biodiversity, European Long-Term Ecosystem Research Network (eLTER), soil microbiome, standard observations;

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Conflicts of interest

The authors have declared that no competing interests exist.

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