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A metagenome-based comparative analysis of the rhizosphere of oak (*Quercus*) and black locust (*Robinia*) forest stands and a comparison of the results with rhizosphere metagenome data from agricultural experiments

Theses of Doctoral (PhD) Dissertation

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Introduction

Man's conscious use of forests, including both afforestation and harvesting, is one of the most intensive landscape-forming activities. These interventions, in addition to significantly affecting the plant and animal biodiversity of a given area (Buscardo et al., 2008; Lencinas et al., 2014), have a significant impact on soil physical and chemical properties (Roy et al., 2021; Segura et al., 2021), but can also cause significant changes in the composition and functioning of the soil microbial community (Smenderovac et al., 2017) Since different vegetation types often show differences in soil microbial community composition and activity due to their different composition and structure (McCulley et al., 2004; Wu et al., 2018), in addition to strong human interventions, natural succession processes and changes in species composition, whether natural or artificial, also influence the composition and activity of the soil bacterial community. This is also true for forest stands with different tree species composition, and the relationship is both indirect (e.g., soil composition) and through direct effects (e.g., root exudate production) (Dukunde et al., 2019). Our present research aimed to increase scientific knowledge on the relationship between forest and soil microbiota.

Aims of the studies

For our soil microbiota composition and activity studies, we selected three planted forest stands: a mixed pedunculate oak stand (ET1) in the regeneration phase, an unmixed black locust stand (EA1) and a mature mixed pedunculate oak stand (ET2). Our results were also compared with metagenomic and microrespiration studies on soil samples from an intensive agricultural experiment and soil samples from an undisturbed, semi-natural fallow.

The main questions of our research were:

- Which taxa constitute the soil bacterial community of each forest stand?
- Are there differences in diversity between soil bacterial communities of forest stands with different structures?
- What forest structural and edaphic factors influence the composition of the bacterial communities in each forest?
- What is the effect of forest stand clear-cutting on the composition of the bacterial community?
- To what extent does the composition of the soil bacterial community in forest stands differ from that of intensively farmed agricultural land and near-natural fallow?
- Are there differences in the patterns of substrate utilization between the soil microbial communities of different forest stands?
- What is the effect of the end-use (clear-cutting) of the forest stand on soil microbial community activity and substrate utilization?
- To what extent does the substrate-induced respiration of soil microbial communities in forest stands differ from that in agricultural areas?

Materials and methods

- In the experimental plots, soil samples were collected on 16 occasions between 2018 and 2021 from soil depths between 0-10 cm (A) and 10-40 cm (B).
- The main soil physical and soil chemical parameters of the soil samples were determined.
- 16S rRNA gene amplicon sequencing was performed using the Illumina MiSeq sequencing platform to analyze the structure of soil bacterial communities.
- The respiratory activity of the soil microbiota was assessed by MicroResp™ substrate-induced catabolic activity analysis.

Summary of the results – thesis points

1. In the soil metagenome analysis of black locust and mixed pedunculate oak stands established on semiarid chernozem soils, which are considered marginal sites for forestry use, we measured the highest bacterial species richness and diversity values in the black locust stand, which is due to the higher organic matter content of the black locust soil. **Thus, the species richness and diversity of the soil bacterial community in the area we studied is determined by the organic matter content of the forest stand soil.**

2. Following the harvesting of the mature mixed oak stand in 2019, we observed a slight increase in species richness in the upper soil depth (0-10 cm) compared to the mixed oak stand with similar species composition already in the regeneration phase and a somewhat more

substantial decrease in diversity and species richness in the deeper soil depth. We attributed the observed changes to rhizodepositional effects due to clear-cutting: in the upper soil depth, the root activity of herbaceous vegetation colonizing the harvested area could have increased the species richness of the soil bacterial community, while in the lower soil depth, the reduced root exudate production due to the clear-cutting of old trees could have limited the species richness and diversity of the bacterial community. **Thus, in our study area, changes in nutrient cycling after harvesting have a dominant effect on the species richness and diversity of the bacterial community in the soil of the forest stand affected by the disturbance.**

3. The soil bacterial community composition of the forest stands we studied showed high similarity with each other, with a dominance of Acidobacteriota, Actinomycetota, Pseudomonatoda, Verrucomicrobiota, Bacteroidota and Gemmatimonadota at the phylum level. **Thus, the differences in soil bacterial community structure in the area studied are due to differences in the relative diversity of the main taxa, of which the role of Acidobacteriota and Actinomycetota is dominant in driving the variation between stands.**

4. Among the environmental variables, soil pH had the most significant effect on the composition of soil bacterial communities in the studied forest stands, whose seasonal and cutting-induced changes had the greatest influence on the relative abundance of the pH-dependent Acidobacteriota strain. **Thus, in our study area, the soil's prevailing physical and chemical properties (mainly soil moisture and total**

carbon, in addition to pH) have a more significant short-term effect on soil bacterial community structure after forest harvest than the cessation of forest cover.

5. In the analysis of the catabolic activity of the soil microbial community in black locust and mixed oak stands established on semiarid chernozem soils, we measured higher average substrate-induced respiration in black locust and mature oak stands. The significant respiration values in the black locust stand could be explained by the higher organic matter content at each soil depth, and in the mature oak stand by the more favourable microclimatic conditions provided by the stand structure before the clear-cutting and the favourable nutrient cycling caused by the developing herbaceous vegetation in the second and third years after the harvest. **In our study area, the respiration activity of the soil microbial community in forest stands is, therefore, significantly influenced by soil nutrient conditions and forest structure.**

6. After the harvest of the mature mixed oak stand (ET2), we observed only a transient substrate-induced respiration decline in the soil samples of the stand and found that the respiration decline was more pronounced at depths of 10-40 cm. In the upper depths of the soil, the decrease in carbon source utilization was most pronounced for carboxylic acids (malate, succinate, citrate), whereas the trend was observed for all major carbon sources at deeper soil depths. **Thus, in our study area, forest clearance causes a marked qualitative change in substrate utilization by microbial communities at soil depths of 0-10 cm and a quantitative change at soil depths of 10-40 cm.**

7. In a catabolic activity analysis of the soil microbial community of black locust and mixed oak stands established on semiarid chernozem soils, soil pH was found to have a prominent effect on the substrate utilization patterns of individual stands. **Thus, in our study area, soil microbial community carbon source utilization (substrate preference) of forest stands is a process closely related to soil pH.**

8. The substrate-induced respiration analysis of the soil microbial community of the forest stands we studied revealed the dominant influence of citrate in the development of inter-stand variance; the use of this carbon source was prominent in the mature mixed oak stand during the study period. **Thus, citrate plays a significant role in shaping the carbon source utilization patterns of forest stand soil microbiome. It can be assumed that the high citrate usage by the soil microbial community of the forest stand affected by the harvesting indicates an essential role of this carbon source in the metabolic processes induced by environmental stress.**

9. Metagenomic and substrate-induced respiration analysis of forest stands with different tree species composition and structure, intensive agricultural crops and undisturbed fallow established on semiarid chernozem soils revealed different soil bacterial community structures but similar soil microbial activity for each management type. **Thus, the soil bacterial community structure of forest stands in our study area is significantly different from the soil bacterial community structure of agriculturally cultivated areas, while the substrate-induced respiration**

pattern of the soil microbial community does not allow to distinguish between different land use types.

Conclusions

Based on our research, we found that

a. Different forest structures (tree species composition, age, canopy closure) favour the development of a soil microbiome with different structures, diversity, and activity.
b. The effect of forest stand clear-cutting on the soil microbiota is most significant in the year of the disturbance.
c. The effect of soil parameters, especially soil pH, is a determinant of soil microbiota composition and activity.
d. The soil microbiota community in forest stands differs from the soil microbiota community in agricultural soils mainly in its structure.

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List of publications related to the PhD thesis

[1] **Bereczki, K.**, Benke, A., Tóth, E.G., Megyes, M., Korponai, K., Szili-Kovács, T., Illés, G., Lados, B.B., and Márialigeti, K., 2024. Soil pH and Nutrient Content Sustain Variability of Soil Bacterial Community Structure and Activity after Forest Clear-Cutting. Forests, 15(8). https://doi.org/10.3390/microorganisms12061162

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