The Occurrence of Microorganisms in Grassland Soils

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Soil microorganisms are responsible for the mineralization of organic compounds and the course of various biogeochemical cycles. They have an influence on the biodiversity of entire ecosystems, including the plant cover. They are responsible for the productivity of soil and its structure, and they also affect the circulation of elements in nature. The count and species composition of soil microorganisms depend mainly on the physicochemical properties of the pedon, the amount of nutrients, the type of soil, and the species composition of the plant community. Plants significantly influence the species composition of soil microbial communities through the release or loss of compounds from plant roots to the surrounding soil environment and decomposition of litter and roots.

grassland use of meadows and pastures bacteria fungi

1. Bacteria

As results from scientific research suggest, the populations of soil bacteria in grasslands used as pastures decrease with the soil depth. Hu et al. ^[1] observed that bacteria were the most abundant microbes found in grassy plots used for free grazing. Their share in the total amount of soil microorganisms exceeded 60% and amounted to 7.35 (\pm 0.44) nmol g⁻¹ DW. The content of Gram-positive bacteria (2.52 (\pm 0.3) nmol g⁻¹ DW) was about half lower than the content of Gram-negative bacteria (4.68 (\pm 0.31) nmol g⁻¹ DW). However, their count tended to decrease along with the depth of the pedon, which was related to the profile distribution of humus. The researchers found that the total count of actinobacteria in the soil of grasslands used for grazing amounted to 0.89 (\pm 0.11) nmol g⁻¹ DW, whereas in the soil of the grasslands that were not used as pastures, the population of these microorganisms was 0.91 (\pm 0.11) nmol g⁻¹ DW, and it was relatively stable up to a soil depth of 30 cm. Significant correlations were found between organic matter, TN, TP, AP, NO₃⁻-N, and total PLFAs, bacteria, G⁺ bacteria, G⁻ bacteria, actinomycetes, and G⁻/G⁺. These results indicate the effect of grass vegetation and changes in soil physicochemical properties on microbial biomass.

The research conducted at the USDA ARS High Plains Grasslands Research Station ^[2] compared the effects of continuous light grazing and continuous heavy grazing. The analysis of the pattern of the content of cellular phospholipids—PLFA (phospholipid-derived fatty acids)—proved that the counts of Gram-positive and Gram-negative bacteria in the intensive grazing system were lower than in the light grazing system. The same dependence was observed in the total bacterial count. Apart from that, Ingram et al. ^[2] and Liu et al. ^[3] noted that, in general, there were more microorganisms in the 0–5 cm soil layer than in the 5–15 cm layer, which was characteristic of steppe areas. It is probably due to the higher root density in the topsoil. Plant roots stimulate

microbial activity by providing them with water-soluble compounds, including organic acids, sugars, or amino acids. It follows that in no other part of the soil will microbial activity be as dynamic as in the root zone. The researchers observed that actinobacteria were the second most numerous group of soil microorganisms, regardless of the intensity of grassland use. As was the case with fungi, the largest communities of actinobacteria were found in light grazing soils, whereas intensive grazing was accompanied by the smallest communities of actinobacteria. The authors of the study noted that light to moderate grazing favors both the maintenance of soil physicochemical properties and the availability of resources for soil biota and processes controlled by them ^[2].

Qu et al. ^[4] found that the diversity of the bacterial population depended on the intensity of grazing and the botanical composition of the sward. The research conducted on grazing grasslands at the Grassland Ecological Research Station of Northeast Normal University (Jilin Province, China) showed that there were larger concentrations of bacteria under specific grass species (*Leymus chinensis, Phragmites australis, Calamagrostis epigeios, Chloris virgata*). Apart from that, the research showed that the lighter grazing system was better for both the diversity of bacteria and the species composition of plant communities. In the intensive grazing system, the indicators of plant biodiversity and the counts of bacterial populations decreased gradually. However, the plant species composition was the key factor influencing the variability of the microbial composition, whereas the intensity of use was of secondary importance. The soil N/P ratio, total nitrogen, and pH also significantly influenced the composition of bacterial communities, which affected the species composition of plant communities growing in a specific pasture.

In a paper synthesizing the results of the responses of soil microbial community size and SR to grazing, it was found that both the moderate and intensive systems of using grassland as pastures had a negative influence on the populations of soil bacteria because their counts decreased by 0.28% and 28.12%, respectively. The analysis of the counts of bacteria and soil depth showed that grazing significantly reduced the microbial communities—by 8.17%, 21.20%, 18.12%, and 69.52% at soil depths of 0–10, 10–20, 20–30, and >30 cm, respectively. The authors emphasized that soil microbial community size can determine soil carbon dynamics under grazing. Furthermore, they indicated that grazing intensity can be useful in predicting soil C [5].

Musiał et al. ^[6] conducted a study on the grasslands located in Brody, at the Experimental Station of the Poznań University of Life Sciences, Poland, and observed that the type of grassland use and the weather conditions significantly influenced the microbiological composition of soil. During the three-year experiment, *Azotobacter* spp. bacteria reacted significantly to the type of grassland use. At the beginning of the first year of the experiment, the count of these bacteria in the soil under mown grassland decreased significantly—during the first mowing cycle, it amounted to 24.7 cfu g⁻¹ DM of soil, whereas the count of these bacteria in the grassland used as a pasture was 55.5 cfu g⁻¹ DM of soil. However, during the research, *Azotobacter* began to react differently to the soil use, and the count of these bacteria changed. At the end of 2006, the *Azotobacter* population was significantly higher under the mown grassland use did not cause significant differences in the total count of bacterial populations. Out of the twelve grassland use cycles during the three years of the study, there were three mowing cycles with higher counts of bacteria—the last cycle in 2006, the first in 2007, and the last in 2008. By contrast,

there were only two cycles with higher counts of bacteria in the grasslands used for grazing. The researchers found that among all microorganisms under analysis, actinobacteria were the most sensitive to the way the plant community was used. The highest abundance of actinobacteria was noted during the fourth mowing cycle combined with grazing. In general, there were larger populations of actinobacteria under the mown grassland. The greatest disproportion was observed during the last mowing cycle in the last year of the study (2008), when the count of actinobacteria under the mown meadows was 102.4 cfu g⁻¹ DM of soil, whereas the count of these microorganisms under pastures amounted to 43.9 cfu g⁻¹ DM of soil. The researchers also noted a high abundance of actinobacteria during dry periods. The authors observed a regularity in the effect of actinobacteria on inorganic N content, irrespective of the sward management system, which significantly underlines the importance of this group of microorganisms in N transformations in muck soil. According to Solecka et al. ^[7], actinobacteria can adapt to climatic conditions (humidity, sunlight, and temperature).

The comparison of the effects of manual and mechanical mowing of meadows showed that the mowing method influenced only the count of proteolytic bacteria. The highest count of these bacteria was found in the soil under unmown grasslands, whereas the lowest count was found in the soil under mechanically mown grasslands. Phosphate solubilizing bacteria reacted in the opposite way. There were greater counts of these bacteria in the soil under the mown meadows than in the unused meadows. The populations of vegetative forms of bacteria and ammonifying bacteria increased after mowing. By contrast, the *Azotobacter* bacteria were more abundant before mowing ^[8]. However, a higher abundance of proteolytic bacteria was found in soil with no mowing, which is most likely due to the slow mineralization dynamics of organic compounds. Kizilova et al. ^[9] reported that in mountain meadow soils, this is due to the high accumulation of C and N in poorly moistened organic matter.

According to Chmolowska et al. ^[10], the mown meadows (in the late summer, most of them are extensively grazed) in the Outer Carpathians (the Beskid Sądecki Mountains) had 47% of bacteria in the total count of soil microorganisms, whereas the share of bacteria under fallow land amounted to 46%. The authors noted that this difference was statistically significant. The fact that the relative PLFA content of the bacteria in the hay meadows was higher than in the fallow meadows may have been caused by the volume of the plant root biomass. The share of actinobacteria in the total count of microorganisms in the soils under the hay meadows in the Outer Carpathians was only about 5%. The researchers indicated that actinobacteria were the main factor affecting the similarity of the communities of soil microorganisms.

Ilmarinen et al. ^[11] conducted a study on the soil microbiome under meadows located in central Finland, which were mown with different frequencies. The researchers observed that the method of use (no mowing, mowing once a year, mowing twice a year) did not have much influence on the bacterial respiration (BR) and substrate-induced respiration (SIR) of soil microbes. BR and SIR are measures of the amount of carbon dioxide released from soil that is released by the decomposition of soil organic matter and plant litter. The difference is that in SIR, glucose is additionally induced into soil samples. These parameters are used to estimate the biomass of soil microorganisms [11][12].

The research conducted by Bei et al. ^[13], at the Environmental Monitoring and Climate Impact Research Station Linden, Germany, showed that the occurrence of soil microorganisms in meadows mown twice a year had a seasonal nature. The authors pointed to the domination of bacteria over other soil microorganisms, and this observation was consistently confirmed by their research. As the seasons of the year changed (winter–summer), the bacterial population increased considerably. However, the α -biodiversity at the genus level remained almost identical between the two seasons.

2. Fungi

According to Hu et al. ^[1], fungi had the smallest share among the entire microbial population analyzed in the study conducted on the grassland located in Taibus Banner in Inner Mongolia. The total count of fungi in the soil of the grasslands used as pastures was 0.75 (\pm 0.24) nmol g⁻¹ DW. The most fungi were found in the upper soil layers. Their count tended to decrease significantly along with the soil depth. The total count of fungi in the soil of the grasslands not used as pastures was 0.75 (\pm 0.24) nmol g⁻¹ DW. The fungal population also tended to decrease with the soil depth, but this trend was less noticeable than in the grasslands used as pastures. The authors indicated a strong and positive correlation of soil water and belowground biomass with fungi.

In the soils belonging to the USDA ARS High Plains Grasslands Research Station, the count of fungi in the top layer amounted to 2.6–3.2 nmol g^{-1} . The highest abundance of fungi was noted in pastures used for light grazing, whereas the lowest was observed in those used more intensively. However, in the grasslands used for more intensive grazing, the fungal population in the lower soil layer (5–15 cm) was slightly greater than in the 0–5 cm layer. In addition, it was found that lightly grazed conditions had higher fungal concentrations and a lower ratio of bacteria to fungi compared to heavily grazed conditions ^[2].

Zhao et al. ^[5] proved that the light and moderate grazing intensity increased the total abundance of fungal communities by 17.01% and 0.80%, respectively, whereas intensive grazing decreased the abundance of these communities by 16.48%. Moreover, the researchers observed that the total count of fungi decreased with the soil depth as follows: 16.64%, 12.96%, and 56.66% at depths 10–20, 20–30, and >30 cm, respectively. However, the abundance of fungal communities in the top layer (0–10 cm) increased by 22.45%. It is worth noting that the authors noted strong correlations between grazing duration and response rates of total microbial community size. Grazing duration was more significant than SOC or TN.

According to Musiał et al. ^[6], despite the high variability during the three-year study period, the abundance of fungi was similarly influenced by the sward management system. In the first year of the experiment, there was a visible response after the third mowing and grazing cycle. The fungi content in the soil under the mown grasslands amounted to 8.6 cfu g⁻¹ DM of soil. It was five times greater than in the soil under the grasslands used as pastures, where it amounted to 1.6 cfu g⁻¹ DM. This trend was also observed in the following years of the research. The authors emphasized that the greatest increase in fungal abundance occurred in response to the expansion of *Dactylis glomerata* in mowed grassland. They explained this by the drying and soaking process, which then creates favorable conditions for the proliferation of mold fungi ^{[14][15]}.

The share of fungi in the hay meadows of the Outer Carpathians amounted to 11% of the total structural biodiversity of the microorganisms analyzed with the PLFA method. The content of arbuscular mycorrhizal fungi ranged from 5.24 nM g dwt⁻¹ to 10.68 nM g dwt⁻¹, depending on the meadow under analysis. The authors suggested that the effect of microbial community structure on higher respiration rates in meadow soils may be related to the assumption that mycorrhiza is involved in total soil respiration ^[10]. Berg and McClaugherty ^[16] suggested that mycorrhizal fungi are responsible for respiration bursts following decomposition of humus.

Ilmarinen et al. ^[11] indicated that regardless of the frequency of mowing Finnish meadows, it had a neutral effect on the population of mycorrhizal fungi. Grassland use by mowing had no effect on the overall AM colonization rate of plant roots nor on the intensity of AM colonization and the abundance of parent fungi in colonized root parts. It may have been caused by the species composition of the meadows and the fungal reaction to defoliation.

The winter and summer analyses of the occurrence of fungi in meadows mown twice a year showed a high diversity of the species composition. According to Bei et al. ^[13], the growth of the fungal population may have been caused by the summer growth of roots followed by arbuscular mycorrhization.

According to Józefowska et al. ^[8], the occurrence of fungi in the meadows mown twice a year in the Carpathians depended on the mowing method and date. The measurements taken in June showed higher values for all mowing methods than the measurements taken in October. In June, the highest count of fungi was measured in the manually mown meadows, whereas the lowest was noted in the mechanically mown meadows. The analyses conducted in October showed the highest count of fungi in the mechanically mown meadows and the lowest in the unmown ones.

The results of the research conducted in the Romanche River valley in the central French Alps show differences between the grasslands used alternately as hay meadows and pastures (mown meadows) and the meadows used for light grazing (unmown meadows). The MPN (most probable number) statistical method was used for the analysis of the area of mown and unmown grasslands. The two types of grassland use resulted in significant differences in the count of mycorrhizal diasporas. The count of the diasporas of arbuscular mycorrhizal fungi in 100 g of soil on the grasslands used in the alternate hay meadow and pasture system was 15 times greater than in the unmown grasslands. However, the authors found that cessation of mowing negatively affected AMF infection potential as well as plant growth. In addition, cessation of mowing resulted in an increase in endophyte infection on leaves and a decrease in mycorrhizal density, which may suggest links between these groups of fungal symbionts [17].

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