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Review

Soil Microbial Community and Enzymatic Activity of Grasslands under Different Use Practices: A Review

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Abstract: The usage of grassland significantly affects the microbial and biochemical parameters of soil epipedons. The use of grasslands (by mowing, grazing, and mowing and grazing) affects the dominance of bacteria in abundance relative to fungal populations. This was particularly noticeable when manual mowing was applied. In general, the highest number of microorganisms occurred during spring and summer, which should be associated with the intensity of growth of root systems of grass vegetation. It was noted that the grazing system caused an increase in the enzymatic activity of urease and slightly less dehydrogenases and acid and alkaline phosphatase. Therefore, microbial abundance and enzymatic activity are considered as indicator parameters to evaluate the biological soil environment. They are highly probable estimates of soil fertility and ecosystem biodiversity.

Keywords: grassland; use of meadows and pastures; bacteria; fungi; enzyme activity

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1. Introduction

Grassland is one of the largest ecosystems in the world. It covers about 3.5 billion hectares (ha), which is 26% of the world's land area and 70% of agricultural land [1]. Meadows and pastures are ecosystems closely related to human activity. Most of them developed as a result of using cleared forest areas for agricultural purposes. Due to the growth of the human population and the dynamic development of civilization, fertile land began to be used for growing crops in fields, whereas marginal lands of lower fertility as well as wetlands were used as meadows and pastures. Their maintenance and use are related to pastoralism and animal husbandry [2].

Grasslands can be divided into natural, semi-natural, and anthropogenic ones. Grasslands used for agricultural purposes for more than 5 years are defined as permanent, whereas those used for a short time on arable lands and in a crop rotation system are defined as alternate lands [3].

The sward of grasslands is dominated by grasses as well as sedges, rushes, dicotyledons, and small shrubs, which can be chewed by grazing animals. The form of grassland use determines the species structure of the sward. Species of tall grasses predominate in hay meadows. On the other hand, species of short grasses predominate in the sward of pastures, whereas the share of selected species of tall grasses is small [2,4]. Apart from grasses, legumes play an important role in the floristic composition of the sward of grasslands. They form symbiotic systems with bacteria of the *Rhizobium*, *Bradyrhizobium*, and *Sinorhizobium* genera, thanks to which they can fix atmospheric nitrogen. Thus, they enrich the soil with nitrogen and contribute to the intensive development of plants and the formation of protein-rich biomass. Apart from that, the grassland sward also includes species of herbs and weeds—the functional group characterized by a higher content of protein, carbohydrates, minerals, vitamins, and other active substances which are beneficial for animal health [2,4,5].

Permanent grasslands can be used as hay meadows and pastures as well as for both of these purposes in an alternating manner. Their species composition depends on the form and intensity of their use [2,6–8]. It affects the quality and quantity of animal feed obtained from these grasslands [9,10].

Apart from being a source of feed, grasslands have many other ecosystem functions, which are important for humans. They have key functions in nature: climatic, hydrological, protective, filtration and phytosanitary, scenic, aesthetic, and biocenotic. Grasslands are a refuge for numerous species of plants and animals, including rare and protected ones [6,11,12].

The richness and floristic diversity of plant communities are mostly influenced by the habitat conditions, including the type of soil and its abundance of nutrients, water conditions, and the presence of soil microorganisms [13,14]. Microorganisms and plant cover determine the nature and direction of biogeochemical processes as well as physicochemical changes occurring in soils [15]. Moreover, they have considerable influence on the fertility of soil and its yield potential as well as the yield of crops [14]. Bioactivity and interactions occurring in the soil environment are the basis of various ecosystem functions, including the circulation of nutrients, pathogen control, water infiltration, and the formation of trophic chains.

Bacteria, which are prokaryotic organisms, easily adapt to a given environment. They widely colonize both natural and anthropogenic environments. Bacteria of the *Azotobacter* spp. and *Azospirillum* spp. genera are particularly important. These free-living soil microorganisms are capable of fixing atmospheric nitrogen and making it available in an absorbable form to higher plants. *Azotobacter* are aerobic bacteria, producing a wide range of compounds, vitamins, and siderophores stimulating the growth and development of plants [16]. They are used as a potential indicator of soil fertility [17]. These microorganisms strongly react to chemical and physical factors in soil. Therefore, fluctuations in their population are a good indicator of changes occurring in the environment. There are seven known species of bacteria of the *Azotobacter* genus, among which *Azotobacter chroococcum* is believed to be the most common in various soils around the world. The abundance of *Azotobacter* in neutral and alkaline soils rarely exceeds several thousand cells per 1 g of soil. These bacteria are almost absent from acidic soils (pH < 6) or occur in them in small amounts [16]. *Azospirillum* spp. bacteria (also widespread around the world) occur in large populations—up to 10^7 cells per g of rhizosphere soil. These bacteria are associated with the roots, stems, and leaves of various plants (mainly cereals and grasses), grown both in temperate and tropical climates [18–20]. This group of bacteria is equally important because they can activate the mechanisms fighting plant pathogens (bioprotectants). Apart from that, they improve the uptake of nutrients (biofertilizers) and are capable of producing plant hormones (biostimulants) [18].

Actinobacteria, which belong to the group of Gram-positive bacteria, are commonly found in soil, composts, water, and bottom sediments. They contribute to the degradation of plant and animal residues as well as hard-to-degrade compounds (lignin, chitin, cellulose, higher fatty acids). They are capable of synthesizing various enzymes, e.g., nitrogenase, as well as substances with antibiotic effects, e.g., erythromycin. Actinobacteria participate in the formation of humic compounds in soil [21]. Organic matter is one of the basic components of soil. It consists of dead plant and animal remains as well as decomposition products and secondary synthesis of humus compounds. The humus positively influences the formation of the aggregate structure of soils, improving water and air relations. In addition, humus influences soil color and, thanks to its dark color, improves soil thermal properties. Organic matter is a source of plant nutrients (mainly nitrogen and phosphorus) and influences the physicochemical properties, e.g., stabilizes the soil pH. Soils rich in organic matter have a higher biological activity [22,23]. Actinobacteria also produce a wide range of secondary metabolites, which play an important role in the soil environment. For example, they improve the availability of fertilizer ingredients and many other micronutrients, thus promoting plant growth [24].

Fungi play a fundamental role in various physiological processes, in the uptake of minerals and water, chemical transformations, and the biosynthesis of biostimulants supporting plants during their exposure to environmental stresses (drought, salinity, contamination with organic and mineral xenobiotics). Plants associated with mycorrhizal fungi are capable of more intensive uptake of nutrients and are better supplied with water, macro- and micronutrients. They are characterized by greater resistance to drought, frost, high temperature, acid rain, heavy metals, and pathogens [20].

Soil enzymes are another important indicator in the ecology of soil microorganisms, as they determine the direction of biochemical changes occurring in soil. They participate in the circulation of elements and play an important role in the synthesis and decomposition of humus, the releasing and supplying of mineral substances to plants, the fixation of molecular nitrogen, detoxification of xenobiotics, nitrification, and denitrification [25,26]. Biochemical reactions occurring in grassland soils are conditioned by various complex factors, which often interact with each other (Figure 1) [27–35].

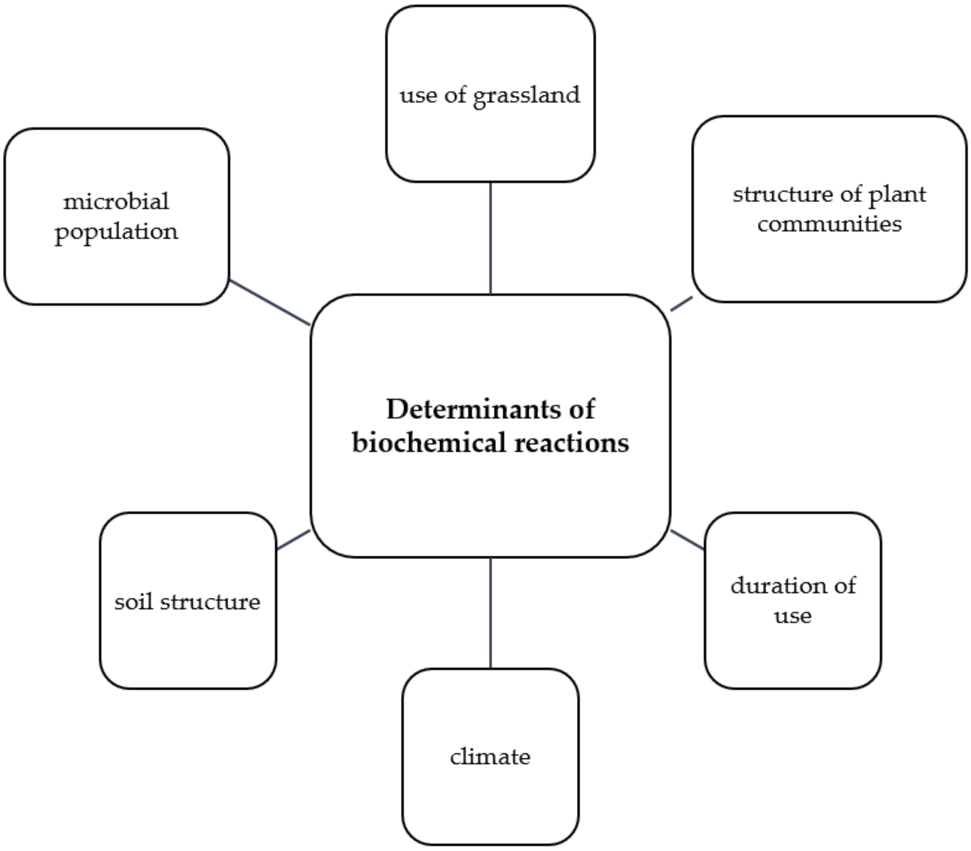

Figure 1. Determinants of biochemical reactions occurring in grassland soils.

Currently, there is a lack of sufficient research on the correlation between grassland usage and biological activity in topsoil in terms of microbiota abundance and activity, especially in Poland. Therefore, this paper focuses primarily on studies conducted in Poland and compares them with results obtained in other countries and explains how various forms of use of meadows and pastures influenced selected biological parameters of superficial soil horizons.

2. Types of Grassland Use

Grassland is characterized by complex interactions between plants and soil, between plants and animals, and between soil and animals [36]. The use of grassland involves the removal of biomass while leaving minimal amounts of plant residues and related nutrients [37].

The direction of use and habitat conditions affect the floristic composition of the sward. According to Tälle [8], in southern Europe, the floristic richness of meadow communities is more influenced by mowing, whereas in Central Europe, it is more influenced by grazing. Some authors [38] indicated that mowing and grazing have similar effects on the number of grassland species. They also observed that when grassland was used for mowing, the Shannon index used for the assessment of biodiversity assumed higher values.

Li et al. [39] found that the yield of the aerial biomass of the sward decreased significantly as the intensity of grazing increased. On the other hand, the highest species biodiversity in the sward was observed when it was moderately used as a pasture. A positive effect of the extensive use of the sward was also observed in the UK [40].

However, it is important to note that, so far, there have been no results of studies on interactions between the direction of use of grassland and its floristic diversity and the biochemical processes occurring in soil as a result of the microbial activity [38].

2.1. Use of Grasslands by Mowing

The biomass harvested from meadows is used for the production of hay or silage with a higher dry matter content. The intensity of use and habitat conditions affect the biodiversity of meadows and their natural and functional value [41,42].

Mowing is an important factor influencing the formation of meadow communities with their characteristic combination of species. The hay meadows should be dominated by tall grasses with high productivity, but there should also be low grasses with strong ground cover and rich foliage [6]. In view of the fact that this treatment is cyclical, the date of the first mowing and the frequency of these procedures have a particularly significant influence on the botanical composition of meadows [6,43].

The date of mowing meadows depends on their species composition. Under the optimal habitat conditions, i.e., with a supply of water and tropism, a high yield of biomass can be harvested from meadows. In view of the fact that the development phase of the dominant grass species, i.e., the beginning of the heading phase, is the main determinant of mowing time, the number of mowings depends on the species composition of the sward. It is noteworthy that under optimal habitat conditions of Central Europe, there may be even as many as three or four mowings. Extensive use of grasslands is recommended, i.e., mowing twice a year, in order to maintain high biodiversity of the sward. However, the number of mowings depends on the sward regrowth rate, which is mainly determined by the water conditions [44].

2.2. Use of Grasslands by Grazing

Grazing also has a significant influence on the sward of grasslands. The pasture is a place where plants and animals interact. By removing biomass, animals affect not only the species composition of the grassland sward but also the physicochemical conditions of soil and its biological characteristics [45,46].

Grazing animals leave their solid and liquid excrements on grazed areas, providing nutrients such as nitrogen, phosphorus, potassium, calcium, and magnesium to the soil. Twardy and Mikołajczyk-Rusin [47] emphasized that rational pasture use makes it possible to reduce the need for mineral fertilizers.

Grazing is one of the factors limiting the intensive processes of decomposition of organic matter and its mineralization on organic soils. The pressure from grazing animals' hooves compacts the topsoil, which promotes the formation of a dense and elastic sward. On mineral soils, however, intensive animal browsing has a negative effect on the physical and chemical properties of the turf, e.g., it reduces the porosity of the soil and thus limits water and air retention [48–50].

The proper botanical composition of grasslands guarantees a diversified fodder base for animals. A good pasture should consist of 30% tall grasses, 50% low grasses, 10–20% legumes, and 10% other dicotyledonous plants, the so-called herbs and weeds group. The most preferable tall grass species are *Festuca pratensis*, *Phleum pratense*, and *Dactylis* *glomerata*, while short grasses are *Lolium perenne, Poa pratensis*, and *Festuca rubra* [51]. As was the case with meadows, the intensity of use of pastures depends mostly on the habitat conditions. The starting date of grazing is determined by the pasture maturity, i.e., when the height of the sward is about 10–12 cm. The number of grazings is determined by the sward regrowth rate, which depends mainly on the climatic conditions. The duration of grazing, which should be short, is an important factor influencing the species composition of the sward. The species diversity of the pasture is influenced by the grazing system, i.e., the quarter (rotational), dosed, free, or continuous system. The dosed system, which is the most intensive, results in a considerable simplification of the sward species composition. Due to the high density of grazing animals, it has the greatest influence on the physical, chemical, and biological properties of soil [2,7] because it enables almost complete eating of the sward. The method of feeding is based on systematic, according to a predetermined grazing schedule, separation of animals from the pasture by means of portable fencing [52].

2.3. Alternate Use of Grassland by Mowing and Grazing

Sometimes, when the weather conditions are favorable, the alternate use of grasslands as hay meadows and as pastures is promoted in order to maintain the right proportion between the tall and short plant species in the sward [53]. According to Kamiński and Chrzanowski [54], alternate use of grasslands by mowing and grazing is characterized by the highest share of tall grasses in the sward, and the remaining species constitute about 25%, which ensures the optimum level of yielding and provides a high quality of forage obtained.

Thanks to this use, it is possible to regulate the floristic composition of the sward and prevent the appearance of undesirable species, i.e., weeds. For example, on fertile soils with a high content of humus and on peat soils, this system of grassland use prevents the development of species of low natural and functional value, such as umbellifers, e.g., *Heracleum sphondylium* and *Aegopodium podagraria*, and complex plants, e.g., *Cirsium palustre* and *Cirsium oleraceum* [54].

Periodic changes in the use of grasslands, i.e., the alternate use of these areas as hay meadows and as pastures, prevent the development of weeds and the loss of species with high forage value. Thanks to this alternate use, it is possible to maintain the species structure of plants and the diversity of the plant community [2,6].

3. The Occurrence of Microorganisms in Grassland Soils

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 [55–57].

3.1. 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. [38] 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 (±0.44) nmol g^{-1} DW. The content of Gram-positive bacteria (2.52 (±0.3) nmol g^{-1} DW) was about half lower than the content of Gram-negative bacteria (4.68 (±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 (±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 (±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, NO_3^--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 [58] 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. [58] and Liu et al. [59] 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 [58].

Qu et al. [30] 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 [60].

Musiał et al. [19] 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 soil under 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 (49.1 cfu g^{-1} DM of soil) than under the grassland used for grazing (29.7 cfu g^{-1} DM of soil). However, the 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. [61], 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 [62]. 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. [63] 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. [64], 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. [65] 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 [65,66].

The research conducted by Bei et al. [67], 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.

3.2. Fungi

According to Hu et al. [38], 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 (±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 (±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 [58].

Zhao et al. [60] 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. [19], 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 [68,69].

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 [64]. Berg and McClaugherty [70] suggested that mycorrhizal fungi are responsible for respiration bursts following decomposition of humus.

Ilmarinen et al. [65] 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. [67], 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. [62], 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 [71].

4. Enzyme Activity in Grassland Soils

The enzyme activity is a sensitive indicator of the function of the ecosystem. It shows what changes take place in the soil and reflects the trends and nature of biogeochemical cycles. By analyzing the enzyme activity it is possible to determine changes in the biological and physicochemical properties of soils [25]. The activity of enzymes depends on their abundance, catalytic efficiency, and the presence of other compounds than enzymes. The catalytic efficiency is affected by the species of soil microorganisms, temperature, pH, the abundance of water and air in soil, and the content of mineral and organic compounds. These parameters are largely stimulated by the way of grassland use [72].

Dehydrogenases (oxidoreductases) are very sensitive to external factors. Therefore, their activity is used as an ecotoxicological indicator. These enzymes play an important role in the transformation of organic carbon. Apart from that, they are a metabolite involved in the production of ATP. Dehydrogenases are very important as their activity levels are taken as an indicator of overall microbial activity due to their intracellular presence in all living microbial cells [26,73].

Phosphatases (hydrolases) are responsible for stimulating the transformation of organic phosphorus compounds into inorganic phosphates, which are an available form of this element to plants and soil organisms. There are acid phosphatase (pH 4–6) and alkaline phosphatase (pH 8–10). The presence of these enzymes in soil is a measure of organic phosphorus mineralization and soil biological activity. Acid phosphatase is secreted mainly by plant roots and fungi. Alkaline phosphatase is a good indicator of the soil pH [26,74]. In addition, plant-growth-promoting endophytes of the genera *Pseudomonas, Bacillus, Enterobacter,* and *Rhodococcus* improve phosphorus assimilation and stimulate plant growth and development. Many endophytic bacteria, by secreting small-molecule organic acids and phosphatases, facilitate phosphate solubilization and thus provide phosphorus to plants [75,76].

Urease (hydrolases) can be found mainly in plant cells and soil microorganisms such as fungi and bacteria. The availability of urease in soil indicates nitrogen transformation.

Therefore, it can be used as a measure of the availability of nitrogen to plants. The level of urease activity shows the level of androgenization of the soil environment because the only factor limiting the activity of this enzyme is the availability of urea in soil [26,77].

Proteases (hydrolases) are responsible for the hydrolysis of peptide bonds. They are mostly found in bacteria and, to a lesser extent, in fungi. There are two main groups of proteases: peptidases, which release extreme amino acids and dipeptide units from proteins and peptides, and proteases (proteinases), which cleave the internal peptide bonds of protein molecules released by microorganisms. These compounds indicate the rate of mineralization of organic nitrogen compounds in soil [26,78].

Catalase (oxidoreductases) protects cells from H_2O_2—their natural substrate which has toxic properties. These enzymes are present in plant and animal cells. Catalase is also produced by many prokaryotic and eukaryotic organisms [26,79,80].

Bielińska et al. [81] analyzed the soils at three research sites—Kąty (PLH060010), Stawska Góra (PLH060018), and Zachodniowołyńska Dolina Bugu (Western Volhynia Bug River Valley) (PLH060035)—and observed differences in the levels of their enzyme activity. The highest enzymatic activity of soil was observed in Gródek, and this was associated with a significantly higher content of total organic carbon and nitrogen. Other authors also indicate that the level of soil enzyme activity is closely related to the content of organic matter in the soil [81–83]. However, in general, the soils where sheep were grazed were characterized by higher activity of dehydrogenases, acid and alkaline phosphatases, and urease than the wasteland soils. In the second year of the research, at one of the sites, the urease activity amounted to 19.40 mg N-NH_4^+ kg^{-1} h^{-1} on wastelands, whereas on pastures, it was 26.38 mg N-NH_4^+ kg^{-1} h^{-1} (Table 1).

Table 1. Effect of grassland use on enzymatic activity.

Use of Grassland	Enzyme	Enzyme Response	Reference
Extensive grazing	Dehydrogenases	increase	[72,81,84–86]
	Alkaline phosphatase	increase	[64,84,87,88]
	Acid phosphatase	increase	[64,72,84,88,89]
	Urease	increase	[64,72,81,84,85,89,90]
	Proteases	no response	[90,91]
	Catalase	decrease	[87,92]
Mowing	Dehydrogenases	increase	[62,86,93,94]
	Alkaline phosphatase	increase	[64,95–97]
	Acid phosphatase	increase	[64,95]
	Urease	decrease	[97]
	Proteases	decrease	[94,98]
	Catalase	increase	[92,94,97]

It may have been caused by the influx of urea (urease substrate) into the soil environment. The main sources of urea (the final product of protein metabolism in terrestrial animals) in the soils on which sheep are grazed are animal excrements, as well as fragments of the tissues and cells of the soil micro-, meso-, and macrofauna, plant residues, and microbial cells [71]. The highest activities of dehydrogenases and acid and alkaline phosphatases were also noted at the same site due to the high content of organic matter. Animal excrements in the soil environment are an additional source of organic matter, macro- and microelements, and an additional source of enzymes secreted by numerous species of bacteria and fungi, which can be found, for example, in sheep urine and feces [85]. Livestock grazing may improve the diversity of herbaceous plants by compacting the soil and thinning out dense groups of trees and shrubs. In addition, animals also contribute to the seed dispersal of many plants; for example, sheep in their wool and on their hooves carry seeds [99]. Bielińska et al. [81] observed a reaction to the change in the grassland use as early as the first year of the research, whereas in the second year, the enzyme activity in the grasslands used as pastures was 1.5 times higher. The differences in the enzyme activity may have been caused by different types of soil and the botanical composition. Due to the fact that vegetation has a diverse species composition and there are different species of bacteria inhabiting plant roots, substrates specific to enzymatic reactions accumulate in soil.

Cui and Holden [90] in their study analyzed, among other things, the effect of grassland stocking rate on the enzymatic activity of soils. The researchers found that this factor had no effect on the protease activity, but it significantly increased the urease activity (Table 1). The greater the animal's density was, the higher the urease activity was because larger amounts of urea, which is the main substrate for this enzyme, were supplied to the soil environment. Apart from that, the researchers found that increased animal density had a positive effect on the activity of nitrogen-related enzymes in both the top and deeper soil layers. On the other hand, the carbon-related enzymes exhibited increased activity only in the deeper soil layers, i.e., 10–20 cm.

The research comparing the enzyme activity in non-grazing soils (grazing finished in one field in 1998 and in the other field in 2002) and in soils used as pastures revealed a significant decrease in the enzyme activity, reflecting the grassland use. The highest enzyme activity was noted in the places where grazing ended earliest (Table 1). The research was conducted at three soil depths. The activity of urease and alkaline phosphatase decreased with the soil depth, but catalase exhibited the highest activity in the middle soil layer, i.e., at 10–20 cm. There were significant differences in the activity of urease and alkaline phosphatase in the topsoil [87].

Chmolowska et al. [64] compared the enzyme activity in wastelands and meadows mown once a year (in the late summer, most of them are extensively grazed) and observed that even though the meadow was mown so rarely, the procedure caused an increase in the enzyme activity. Acid and alkaline phosphatases and urease reacted positively to mowing and light grazing (Table 1). Urease exhibited the greatest difference in the activity level, whereas alkaline phosphatase exhibited the smallest difference in the activity level.

According to Futa et al. [84], the use of grassland as pastures significantly increased the activity of soil enzymes. The researchers analyzed soils used as pastures for horses, sheep, and both horses and sheep in the same year. For sheep, the soil pH was 6.17–7.17 and for horses pH = 6.96–7.35, which did not significantly affect the enzymatic activity of the tested soils. The soils on which horses were grazed exhibited the highest enzyme activity. It was two to three times greater than in the meadow which was not used as a pasture. Sheep grazing resulted in the lowest activity of soil enzymes. Among the three enzymes under analysis, urease exhibited the highest activity, followed by dehydrogenases and phosphatases (Table 1). The research revealed a correlation between the activity of enzymes and the content of organic matter (organic carbon and total nitrogen) in the soil. The researchers noted lower enzyme activities in the second year of the study, which may have been caused by the humidity of the environment (lower rainfall) and the seasonal dynamics in the content of soil nutrients.

The research comparing the enzyme activity in sheep pastures with different conditions of plant communities revealed significant differences in the activity of dehydrogenases and urease, as compared with the control soils. The activities of these enzymes both in the sward with stable plant communities and the swards with disturbed plant development were 1.5 times higher than in the control samples (Table 1). There was a high acid phosphatase activity in the soils with stable plant development, which is characteristic of the roots of young plants [72].

According to Józefowska et al. [62], the method of mowing the meadow (hand mowing with a scythe and mechanical mowing with a lawnmower) and the way in which it is done affects the content of dehydrogenases in the soil (Table 1). The researchers observed that both the activity of microbial biomass carbon (MBC) and the dehydrogenases activity

(DHA) were generally higher in the autumn. Apart from that, the research showed that the mowing method significantly influenced the ratio of dehydrogenases to MBC and total organic carbon. Contrary to the count of microorganisms, the highest enzyme activity was observed when mechanical mowing was applied, both in the summer and autumn.

Herold et al. [88] observed that the microbial biomass and its structure as well as the enzyme activity depended on the soil parameters to a greater extent than on the way the meadows were used.

It is not easy to assess the quality of grassland soils due to the complexity of the soil environment and, above all, due to the variability of its conditions. Numerous studies have shown that grazing has a positive influence on the biochemical parameters of the soil environment. Nevertheless, further long-term monitoring is necessary for a complete, reliable, and objective characterization of the environmental processes occurring in permanent grasslands.

5. Conclusions

The main reason for the change in microbial diversity was the intensity of use and the way grasslands are used. Light grazing and mowing may increase the population of microorganisms, whereas intensive grazing has a negative influence on their abundance and biodiversity. Bacteria are the most numerous population of soil microorganisms regardless of the way meadows are used (intensity of use). The second most numerous group of microorganisms are fungi. Free grazing and mowing increase the activity of enzymes responsible for the metabolism of soil organic matter.

The variety of functions that grasslands perform is related to the principles of sustainable management (water conservation, food production, environmental protection), which is extremely important in times of rapid climate change. Therefore, protective measures and legal regulation of grassland use should be taken into consideration to preserve it in the best possible condition. It is necessary to limit very intensive grazing, which sterilizes soil, and instead promote light grazing and mowing, which stimulate both soil and plant biodiversity. The natural and economic conditions of the area have a great influence on the structural differentiation and change in the way the land is used. In recent years, there has been a steady and systematic decrease in the area used as grassland. This phenomenon should cause concern among both farmers and other social groups and should be monitored.

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