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Impact of Extensive Sheep Grazing on the Biochemical Status of Soils of the Grassland Habitat of Natura 2000

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ABSTRACT

Abandonment of the use of agricultural areas, especially low-production grass communities, and the related secondary succession of trees and shrubs, affects the transformation of the soil environment. The work focused on the impact of extensive sheep grazing on activity of dehydrogenases (DhA) and neutral phosphatase (PhA) and the resources of total organic carbon (TOC) and available phosphorus (Pav) in the soils of meadow habitats in eastern Poland. These habitats are located within the ecological network Natura 2000: PLH060018 Stawska Góra, PLH060035 Zachodniowołyńska Dolina Bugu, PLH060010 Kąty and the Nature Reserve "Kózki" (PLB140001 Dolina Dolnego Bugu and PLH140011 Ostoja Nadbużańska). Soil material was collected for three years, twice each year: before and after the grazing. In the soils of all the studied habitats, a beneficial effect of extensive sheep grazing on soil enzymatic activity and TOC and Pav content was noticed. A particular marked improvement was observed in the biochemical status of the soil in the study area where sheep grazing was continued since 2008. This confirms the hypothesis that free grazing of livestock has a positive impact on biodiversity and the condition of the soil environment of Natura 2000 habitats.

Keywords: dehydrogenases, neutral phosphatase, total organic carbon, available phosphorus, xerothermic grasslands, Natura 2000 ecological network.

INTRODUCTION

Compared to other European countries, Poland is characterized by high biodiversity, both in terms of the number of species and ecosystems, and a varied landscape. The high values for the areas of natural importance and unique environmental potential are testified by the significant percentage of Natura 2000 areas in the territory of Poland. Currently, the Natura 2000 network covers about 20% of the Polish land area, which is slightly higher than the European average (17.5%) (Environment, 2021; OECD, 2023). The Natura 2000 network consists of a system of sites connected by ecological corridors and managed in such a way as to allow the migration, distribution and exchange of the gene pool of species. It is one of the world's largest networks of protected areas (PAs) (Hermoso et al., 2019). Natura 2000 sites are not under strict protection and human activities are allowed. As a result, a significant part of this valuable land is privately owned.

High biodiversity is usually associated with low agricultural productivity. Most of the agricultural areas forming a part of the Natura 2000 network are located in marginal agricultural areas (Oppermann et al., 2012; Toivonen et al., 2013; European Commission, 2019; Möckel 2022). In the past, these areas were quite often grazed due to the poor financial situation of the farmers, or when better pastures were unavailable (Barańska et al., 2013) In places where animals have been grazed for many years, valuable semi-natural habitats have developed, e.g. thermophilic plants (Kulik et al., 2017; 2020). Currently, a significant number of farmers assess the value of these areas through the economic lens. As a consequence, these habitats are usually not used by their owners. The abandonment of the use of naturally valuable Natura 2000 habitats, the lack of grazing and the accumulation of dead organic matter contribute to the formation and intensification of secondary succession to woody shrub communities and forests. This leads to the depletion of phytocoenosis biodiversity (Warda et al., 2016; Steinshamn et al., 2018). As a result, many valuable grassland habitats which are protected under the Natura 2000 network laws could become extinct in Europe (Kulik, 2014; Kulik et al., 2017). One way to prevent these adverse changes is to introduce free grazing of livestock (Barańska et al., 2013; Bielińska et al., 2017). Removal of biomass by animals increases the availability of light in the sward and contributes to the preservation of biodiversity of semi-natural habitats (Rysiak et al., 2021; Chabuz et al., 2019). Free grazing makes it possible to optimize economic and protective activities at a low cost (Gruszecki et al., 2011; Weiss et al., 2013).

Soil plays an important role in the preservation of habitat biodiversity (De Deyn and Kooistra, 2021). It shapes the character of habitats and the natural plant communities growing on them. It is the basic component of the ecosystem that determines the growth capacity of vegetation. The abandonment of the use of agricultural land, especially low-production grasslands, and the related secondary succession affects the transformation of the soil environment (Gruszecki et al., 2011; Futa et al., 2016). The quality of the soil is shaped primarily by the transformation of organic matter, which is mainly associated with microorganisms and the enzymes they secrete (Mencel et al., 2022). All nutrient transformations in the soil are stimulated by enzymes and condition their transition into forms available to plants. These transformations are a key stage limiting the assimilation of nutrients by biocenosis. In order to better understand the C and P cycles, it is important to understand the relationship between soil biological activity and soil processes. Soil enzyme activity (SEA) is considered to be one of the most sensitive indicators of the functioning of an ecosystem, and soil fertility and quality (Lemanowicz, 2015). Therefore, their activity in soil can be considered as a potentially sensitive indicator of changes in the soil environment resulting from such things as livestock grazing (Futa et al., 2016).

The aim of the study was to assess the impact of extensive sheep grazing on the biochemical properties of the soils of grassy habitats in eastern Poland, located within the Natura 2000 ecological network: PLH060018 Stawska Góra, PLH060035 Zachodniowołyńska Dolina Bugu, PLH060010 Kąty and in the Nature Reserve "Kózki" (PLB140001 Dolina Dolnego Bugu and PLH140011 Ostoja Nadbużańska). The research hypothesis was adopted that the introduction of free grazing of livestock to areas where it was abandoned in the past due to a change in the management model will increase the enzymatic activity of soils, dehydrogenases (DhA) and neutral phosphatase (PhA) as well as organic carbon (TOC) and available phosphorus (Pav) resources. It was assumed that the project will have a positive impact on the biodiversity of the soil environment of valuable natural areas.

MATERIALS AND METHODS

The research covered soils of grassland habitats of eastern Poland within the Natura 2000 ecological network: PLH060018 Stawska Góra, PLH060035 Zachodniowołyńska Dolina Bugu, PLH060010 Kąty and in the Nature Reserve "Kózki" (PLB 140001 Dolina Dolnego Bugu and PLH140011 Ostoja Nadbużańska), (Figure 1; Table 1). The habitats are not used by farmers for economic reasons. This is due to the unfavourable location in the relief or the poor quality of the soils.

The soils of the study area differed in terms of typology and grain size (Table 1), and habitat (Table 2). According to the reference list of threats, pressures and actions of the European Commission Directorate-General for the Environment (DG ENV) (https://natura2000.eea.europa.eu), significant threats to the tested xerothermic grasslands (Table 2, Figure 1) are a lack of agricultural use and livestock grazing, biocoenotic evolution, and accumulation of dead organic matter. This leads to strong succession changes in the habitat. On the other hand, the group of medium-level threats includes agricultural activity, i.e. the use of fertilizers

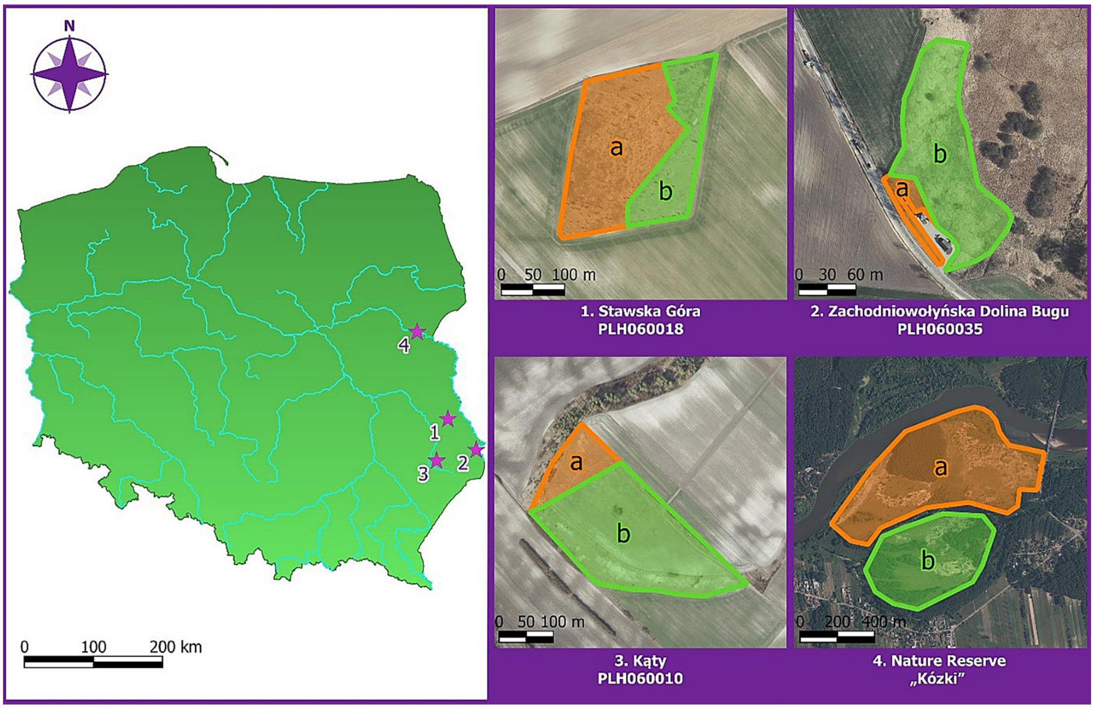

Figure 1. Location of research areas against the background of the administrative division of Poland; (a) ungrazed area; (b) grazed area

Table 1. Texture, particle size distribution and types of soil (IUSS 2015)

Form of protection	Soil texture	Particle size distribution	Soil type
PLH060018 Stawska Góra	Clay loam	2.0-0.05 mm fraction – 29%; 0.05-0.002 mm fraction – 43%; <0.002 mm fraction – 28%	Calcaric Leptosols
PLH060035 Zachodniowołyńska Dolina Bugu	Silt loam	2.0-0.05 mm fraction – 22%; 0.05-0.002 mm fraction – 70%; <0.002 mm fraction – 8%	Albic Luvisol
PLH 060010 Kąty	Sandy clay loam	2.0-0.05 mm fraction – 67%; 0.05-0.002 mm fraction – 12%; <0.002 mm fraction – 21%	Calcaric Leptosols
Nature Reserve „Kózki"	Loamy sand	2.0-0.05 mm fraction – 85%; 0.5-0.25 mm fraction – 10%; <0.002 mm fraction – 5%	Brunic Arenosols

and plant protection products on the farmland adjacent to grasslands. Interspecies competition, hiking which causes trampling of grasslands, plundering of sites and littering have the least impact on grasslands (Kulik et al., 2017; 2019).

The grazing on the selected areas was carried out by native breeds of sheep: świniarka sheep and uhruska sheep, which are well adapted to relatively harsh and seasonally variable living conditions. On the initiative of the "Bocian" Nature Society, in 2008 sheep feeding was started in the Nature Reserve "Kózki". Grazing was continued on the remaining research areas since 2015. In the area intended for grazing, quarters were separated by means of a portable fence. Shelters were also erected to protect the animals during adverse weather conditions, as well as against predators at night. The animals had constant access to fresh water. The degree of turf chewing was also controlled. The load on the animals on the surfaces was in line with nutritional welfare assumptions (Barańska et al., 2013; Lipiec et al., 2015).

The object of the study were the soils of a grazed area and a control area (without grazing). The research was carried out between 2016 and 2018. Soil material for laboratory tests was collected from the 0–20 cm layer under stable weather conditions. Fieldwork was carried out before and after the end of grazing, i.e. in May and October each year. Five soil samples were taken from

Table 2. Characteristics of the research area (own elaboration based on Kulik et al. 2017; 2019)

Location	Form of protection	Habitat (code)
23º40'30"E; 51º20'69"N	PLH060018 Stawska Góra	Flowery xerothermic grasslands (6210-3)
50º47'04"N; 23º56'48" E	PLH060035 Zachodniowołyńska Dolina Bugu	Semi-natural dry grasslands on calcareous substrate (6210)
23º12'46"E; 50º67'52"N	PLH 060010 Kąty	Semi-natural dry grasslands on calcareous substrate (6210)
52º21'36" N; 22º51'36" E	Nature Reserve „Kózki"	Xeric sand calcareous grasslands (6120)

each study area and averaged. Each sample was assayed in three replications.

The activity of two soil enzymes, i.e., dehydrogenases and neutral phosphatase, was determined. The activity of dehydrogenases (DhA) was determined by Thalmann's (Schinner et al., 1995) method using a 1% solution of TTC (2,3,5-triphenyl tetrazolium chloride) as a substrate. Determination of neutral phosphatase activity (PhA) was performed according to Tabatabai and Bremner (Schinner et al. 1995) using a 0.8% solution of pNPP (disodium *p*-nitrophenyl phosphate) as a substrate in buffer pH 6.5. DhA and PhA activity determined colorimetrically, at wavelengths of, respectively: $\lambda = 485$ nm and $\lambda = 410$ nm using a spectrophotometer CECIL CE 2011 (Cecil Instruments, UK). The chemical analyses consisted in determining the following parameters: pH, the content of total organic carbon (TOC), and available phosphorus form (Pav). The pH of the soil was determined at a 1 $mol\cdot dm^{-3}$ KCl using the potentiometric method with a pH-meter (ELMETRON, Poland). The content of TOC was investigated in the TOC-VCSH apparatus with an SSM-5000A module (Shimadzu Corp., Japan). The content of Pav was determined colorimetrically with the use of the Egner-Riehm method in an accredited laboratory.

The statistical analysis of the results was performed using Microsoft Office Excel 2019 and Statistica PL 14 (TIBCO Software Inc., US). Descriptive statistics included the calculation of arithmetic means for individual variants. Statistical evaluation of the variability of the results was carried out using the two-way analysis of variance (ANOVA) method. The significance of the differences between the mean values was verified on the basis of the t-Tukey test. For the studied parameters, the value of Pearson's linear correlation coefficient (r) was calculated, with a significance level of $p < 0.05$. In the study, a maximum of 5% dispersion between measurements in chemical analysis was assumed. The overall similarity between the individual parameters was determined by Ward's cluster analysis and the results were presented in the form of dendrograms.

RESULTS

During the period of the study, the monitored soils were characterized by acidic (Nature Reserve "Kózki"), neutral (PLH060035 Zachodniowołyńska Dolina Bugu) and alkaline (PLH060018 Stawska Góra and PLH060010 Kąty) (Table 3). The observed differences in soil acidity were related to such factors as their typological diversity, grain size and host rock properties (Table 2). The soils of the livestock plots showed statistically significantly higher pH values than the soils of the area outside the grazing range (Table3). In autumn, the pH values of the soils were lower compared to the spring term. Statistically significant differences were observed in the soils of the grazed and ungrazed objects located in the PLH060018 Stawska Góra habitat, pastures in the area of the PLH 060010 Kąty refugium and the Nature Reserve "Kózki". In the years 2016–2018, regardless of the use of the facility, an increase in the pH value of the soils of the tested surfaces was observed over time. However, statistically significant differences were recorded in 2018. These dependencies did not apply to the soil in the Nature Reserve "Kózki".

Explanation: The lowercase letters among different experience factors indicate the significance of difference. The same letters show no significant difference, and different letters show significant difference ($p \leq 0.05$).

The content of organic carbon (TOC) in the soils of the grazed plots ranged from 4.20 to 35.59 $g\cdot kg^{-1}$ and from 3.72 to 34.84 $g\cdot kg^{-1}$ in the soils of ungrazed plots (Table 4). Free grazing of sheep had a beneficial effect on the amount of TOC in the soils of the grazed plots compared to ungrazed plots. However, statistically significant

Table 3. Effect of the interaction of the experimental factors and pH in soils

Experience factors			Form of protection			
			PLH060018 Stawska Góra	PLH060035 Zachodniowołyńska Dolina Bugu	PLH 060010 Kąty	Nature Reserve "Kózki"
Habitat use		grazed	7.39a	7.11a	7.44a	5.06a
		ungrazed	7.26b	6.93b	7.34b	4.69b
Sampling date	grazed	spring	7.46a	7.13a	7.53a	5.09a
		autumn	7.30b	7.09a	7.35b	5.02a
	ungrazed	spring	7.32a	6.97a	7.38a	4.69a
		autumn	7.19b	6.89a	7.31a	4.67a
Years	grazed	2016	7.30a	7.04a	7.31a	5.03a
		2017	7.34a	7.09a	7.39a	5.04a
		2018	7.51b	7.20b	7.60b	5.10a
	ungrazed	2016	7.20a	6.85a	7.24a	4.64a
		2017	7.21a	6.90a	7.26a	4.70a
		2018	7.37b	7.03b	7.52b	4.71a

Note: The lowercase letters among different experience factors indicate the significance of difference. The same letters show no significant difference, and different letters show significant difference ($p \leq 0.05$).

Table 4. Effect of the interaction of the experimental factors and TOC in soils (in $g \cdot kg^{-1}$)

Experience factors			Form of protection			
			PLH060018 Stawska Góra	PLH060035 Zachodniowołyńska Dolina Bugu	PLH 060010 Kąty	PLH060018 Stawska Góra
Habitat use		grazed	28.00a	35.59a	16.54a	4.20a
		ungrazed	27.38a	34.84a	16.07a	3.72b
Sampling date	grazed	spring	28.40a	38.25a	17.12a	4.46a
		autumn	27.45a	32.92b	15.96b	3.93b
	ungrazed	spring	27.80a	37.35a	16.52a	3.87a
		autumn	26.97a	32.30b	15.61b	3.56b
Years	grazed	2016	26.09a	31.87a	15.54a	4.11a
		2017	26.90a	39.30b	16.99b	4.41a
		2018	30.81b	35.59c	17.07b	4.06a
	ungrazed	2016	25.51a	31.67a	15.54a	3.58a
		2017	25.98a	38.59b	16.48b	3.81a
		2018	30.66b	34.19c	16.84b	3.75a

Note: As in table no. 3.

differences were found only in the soil of the area located in the Nature Reserve "Kózki". The obtained results indicate an increase in the fertility of poor sandy soils in this area. This is a positive effect of several years (since 2008) of grazing of świniarka sheep carried out in order to protect the valuable dry grass formations and bare sands of this habitat. In the spring period, the TOC content in soils was generally significantly higher compared to the autumn term. Statistically significant differences were not observed only in the case of the PLH060018 Stawska Góra habitat (Table 4). TOC resources in habitat soils varied in individual years of the study, which can be considered as an indicator of the time-varying intensity of decomposition and accumulation of organic matter. The content of TOC in the soils of the PLH060018 Stawska Góra and PLH060010 Kąty habitats, regardless of the method of development of the study areas, was systematically increasing over the years of research. On the other hand, in the soils of the PLH060035 Zachodniowołyńska Dolina Bugu and the Nature Reserve "Kózki", the highest amounts of TOC were recorded in 2017.

The content of the available form of phosphorus (Pav according to Egner-Riehm) ranged from

1.16 to 27.59 mg·kg^{-1} in the soils of the grazed objects and from 1.04 to 19.26 mg·kg^{-1} in the soils of ungrazed objects (Table 5). A beneficial effect of sheep grazing on the content of available phosphorus was found in the soils of all study plots, which could be related to the excretion of excrement by animals. In the spring period, the content of Pav in the soils of all objects was significantly higher compared to the autumn term. In the three-year study period, a systematic increase in the content of Pav available only in the grazed soil in the area of PLH060018 Stawska Góra was observed.

The efficiency of dehydrogenases (DhA) ranged from 11.94 to 48.59 mg TPFkg^{-1}·24h^{-1} in the soils of grazed area and from 1.23 to 40.96 mg TPFkg^{-1}·24h^{-1} in soils of ungrazed area (Table 6). Regardless of the way the habitat is used, the highest DhA was recorded in the soil of the PLH060035 Zachodniowołyńska Dolina Bugu, and the lowest in the Nature Reserve "Kózki".

DhA in the soils of all the grazed objects was significantly higher compared to the ungrazed soils. Noteworthy is the habitat of dry sand grasslands in the Nature Reserve "Kózki", where about ten times higher activity of dehydrogenases was observed in the soil of the grazed plot compared to the ungrazed plot (Table 6). Regardless of the method of use, it was shown that in the spring period, compared to the autumn, DhA was statistically significantly higher in the soils of the PLH060010 Kąty and PLH060035 Zachodniowołyńska Dolina Bugu habitats. Statistically significant differences were not observed in the case of ungrazed soils in the area of PLH060018 Stawska Góra and in the grazed soils in the Nature Reserve "Kózki" (Table 6). DhA in the soils of the PLH060035 Zachodniowołyńska Dolina Bugu, PLH060018 Stawska Góra and PLH060010 Kąty habitats decreased over the years of research, regardless of the method of habitat management (Table 6).

PhA ranged from 115.57 to 202.91 mmol PNPkg^{-1}·h^{-1} in the soils of the grazed areas and from 85.42 to 176.29 mmol PNPkg^{-1}·h^{-1} in the soils of ungrazed areas (Table 7). As with DhA, livestock use of habitats had a positive effect on PhA. The PhA in the soils of all the grazed objects was generally significantly higher compared to the ungrazed objects. Only within the PLH060018 Stawska Góra habitat no statistically significant differences were recorded. The soil on the grazed plot in the Nature Reserve "Kózki" was characterized by nearly twice as high PhA compared to the ungrazed object (Table7). Regardless of habitat use, soil PhA was generally higher in the fall period compared to the spring term (Table 7). During the study period (2016–2018), PhA varied from object to object. A significant increase in PhA over the years of research was observed only in the area of the PLH060010 Kąty habitat. Whereas, in the soils of other objects, the lowest PhA was recorded in 2018 (Table 7). On the basis of the correlation analysis, it was shown that DhA in the ungrazed areas was closely related to the physicochemical properties of soils, i.e. pH and TOC. Whereas, in

Table 5. Effect of the interaction of the experimental factors and content of available phosphorus (Pav) in soils (in mgkg^{-1})

Experience factors			Form of protection			
			PLH060018 Stawska Góra	PLH060035 Zachodniowołyńska Dolina Bugu	PLH060010 Kąty	Nature Reserve "Kózki"
Habitat use		grazed	24.38a	13.77a	27.59a	1.16a
		ungrazed	10.25b	7.45b	19.26b	1.04b
Sampling date	grazed	spring	26.19a	15.51a	29.42a	1.47a
		autumn	22.57b	12.02b	25.75b	0.85b
	ungrazed	spring	13.70a	9.09a	20.72a	1.28a
		autumn	6.79b	5.80b	17.80b	0.79b
Years	grazed	2016	20.58a	14.67a	27.03a	1.11a
		2017	24.87b	13.29a	28.49a	1.24a
		2018	27.69c	13.35b	27.25a	1.12a
	ungrazed	2016	9.67a	7.21a	19.39a	1.08a
		2017	10.15a	7.61a	19.11a	1.12a
		2018	10.92a	7.54a	19.27a	0.91a

Note: As in table no. 3.

Table 6. Effect of the interaction of the experimental factors and DhA in soils (in mg TPF kg^{-1}·24 h^{-1}).

Experience factors			Form of protection			
			PLH060018 Stawska Góra	PLH060035 Zachodniowołyńska Dolina Bugu	PLH060010 Kąty	Nature Reserve “Kózki”
Habitat use		grazed	16.35a	48.59a	26.18a	11.94a
		ungrazed	12.02b	40.96b	19.96b	1.23b
Sampling date	grazed	spring	13.21a	54.13a	31.43a	12.56a
		autumn	19.48b	43.06b	20.93b	11.31a
	ungrazed	spring	11.81a	48.58a	22.37a	0.57a
		autumn	12.23a	33.33b	17.55b	1.89b
Years	grazed	2016	29.62a	58.12a	43.97a	12.47a
		2017	11.88b	53.89b	24.58b	19.68b
		2018	7.53c	33.77c	9.99c	3.65c
	ungrazed	2016	20.73a	51.32a	30.28a	0.14a
		2017	9.96b	49.13b	23.31b	1.78b
		2018	5.37c	22.42c	6.30c	1.77b

Note: as in Table no. 3.

the grazed areas, only TOC formed DhA. PhA was positively correlated with pH, TOC, and Pav regardless of grassland use (Table 8). In order to determine the similarity of the soils of the studied habitats, cluster analysis was performed. The results of DhA and PhA determinations in the soils of the grazed and ungrazed sites were used as the input data for the analysis of the overall enzymatic activity. Dendrograms showing clusters of objects with similar overall enzymatic activity of soils (Figure 2A and Figure 2B) and soil grain size (Figure 3) were made using the Ward method. An analysis of dendrograms for the overall enzymatic activity soil of ungrazed area (Figure 2) and soil grain size (Figure 3) showed two groups of similar objects: (1) PLH060018 Stawska Góra and PLH060035 Zachodniowołyńska Dolina Bugu and (2) Nature Reserve “Kózki” and PLH060010 Kąty. This proves that the grain size of soils has a significant impact on the level of their biological activity in unused areas. Whereas, the analysis of clusters for the grazed objects showed that the most similar in terms of the overall activity of enzymes in soils were the PLH060018 Stawska Góra and the PLH060035 Zachodniowołyńska Dolina Bugu (Figure 2B). The obtained results support the thesis that grazing has an impact on the biochemical properties of soils.

Table 7. Effect of the interaction of the experimental factors and PhA in soils (in mg mmol PNPkg^{1}·h^{-1})

Experience factors			Form of protection			
			PLH060018 Stawska Góra	PLH060035 Zachodniowołyńska Dolina Bugu	PLH060010 Kąty	Nature Reserve “Kózki”
Habitat use		grazed	187.62a	202.91a	115.57a	160.80a
		ungrazed	164.81a	176.29b	91.73b	85.42b
Sampling date	grazed	spring	171.29a	196.54a	100.09a	180.80a
		autumn	203.95b	209.41b	131.06b	140.80b
	ungrazed	spring	152.30a	160.28a	81.97a	87.32a
		autumn	177.32a	192.30b	101.49b	83.51a
Years	grazed	2016	226.81a	197.42a	85.17a	171.66a
		2017	215.08a	230.10b	101.32b	199.18b
	ungrazed	2016	203.14a	175.01a	81.32a	108.09a
		2017	181.76a	200.98b	84.97a	80.56b
		2018	109.51b	152.89c	108.88b	67.60c

Note: As in Table no. 3.

Table 8. Values of simple correlation coefficients ($y = a + bx$) between the studied enzyme activity and physicochemical properties of soils

Decsription	DhA	PhA	DhA	PhA
	grazed		ungrazed	
pH	n.s	0.50*	0.45*	0.78***
TOC	0.55**	0.50*	0.67***	0.74***
Pav	n.s	0,43*	n.s	0,49*

Note: *** $p \leq 0.00$; 1, ** $p \leq 0.01$; * $p < 0.05$; n.s. – not statistically significant.

DISCUSSION

Soil is the basic component of the habitat that determines the growth capacity of the vegetation. The nature and condition of habitats are shaped by the properties of soils. The properties of the soils of the selected Natura 2000 habitats varied depending on their location, use, timing and years of research. The obtained data and statistical analyses show that in the areas covered by the study, there is a close mutual relationship between the activity of dehydrogenases (DhA) and neutral phosphatase (PhA) and physicochemical parameters. Similar results were obtained by other authors, e.g. Błońska et al. (2017). The types and kinds of soil were also a decisive factor (Table 1), which results, among other things, from different origins and vegetation cover (Table 2), and the direction and severity of the observed changes in DhA and PhA depended on their individual properties (Nannipieri et al., 2018).

During the period of the observations DhA and PhA within all habitats were at a significantly higher level in the soils of the grazed area. This was confirmed by the cluster analysis (Figure 2). The stimulation of the SEA subjects was primarily associated with a higher content of organic matter in the soil of the grazed plots. According to many authors (including Qin et al., 2015; Błońska et al., 2017; Lasota et al., 2021), the SEA levels are closely related to the amount of TOC in soils. The present study supports this thesis, as a significant correlation has been shown between Dh A and PhA and TOC content (Table 8). In addition, PhA was positively and significantly correlated with pH and Pav (Table 8). This shows that the influx of nutrient-rich livestock manure significantly stimulates SEA. The excrement of grazing animals has a strong effect on nutrient concentrations (Carpinelli et al., 2020), shapes the pH (Vilela et al., 2020) and microbial abundance and enzyme activity (Futa et al., 2016), causing changes in the process of decomposition of organic matter and the availability of TOC (Cao et al., 2019).

An increase in the pH value of soils in the grazed areas was demonstrated. One of the main factors in the alkalinisation of the soil was the inflow of urine and sheep excrement, which are abundant in alkaline elements (Ano and Ubochi, 2010; Vilela et al., 2020). Similar results were obtained by, for instance: Ajorlo et al. (2011), Hao and He (2019), and Futa et al. (2021). The increase in TOC recorded in the soils of the grazed plots was also due to the inflow of fresh organic matter in the form of animal manure (Li et al., 2011, Díaz de Otálora et al., 2021). An important factor modifying TOC in soils is also the degree of development and species composition of the vegetation cover as well as the chemical composition of the decomposing organic material (Kulik et al., 2013). According to Wade et al. (2022), grazing increased the TOC content in the topsoil (0–5 cm), while Chen et al. (2021) showed an increase in TOC resources in the soil layer of 0–30 cm. Brewer and Gaudin (2020) showed that livestock grazing can improve soil quality and health by increasing organic matter content. Cellulose and lignin compounds digested by sheep are a source of organic carbon excreted in faeces. This promotes the accumulation of TOC in the soil, which is confirmed by the work of other authors (Galindo et al., 2020; Díaz de Otálorai et al.,2021; Teutscherová et al.,2021).

An increase in pH is usually a factor that stimulates the activity of most soil enzymes, including intracellular dehydrogenases (Bueis et al., 2018) and phosphatases (Lemanowicz 2018). The soil pH can significantly affect enzyme content in the soil environment through the modification of the ionic active form of enzymes, three dimensional structure of enzymes, and substrate affinity to the enzyme (Błońska et al., 2017; Lasota et al., 2021). In the presented studies, no correlation of pH and DhA was observed within the grazed

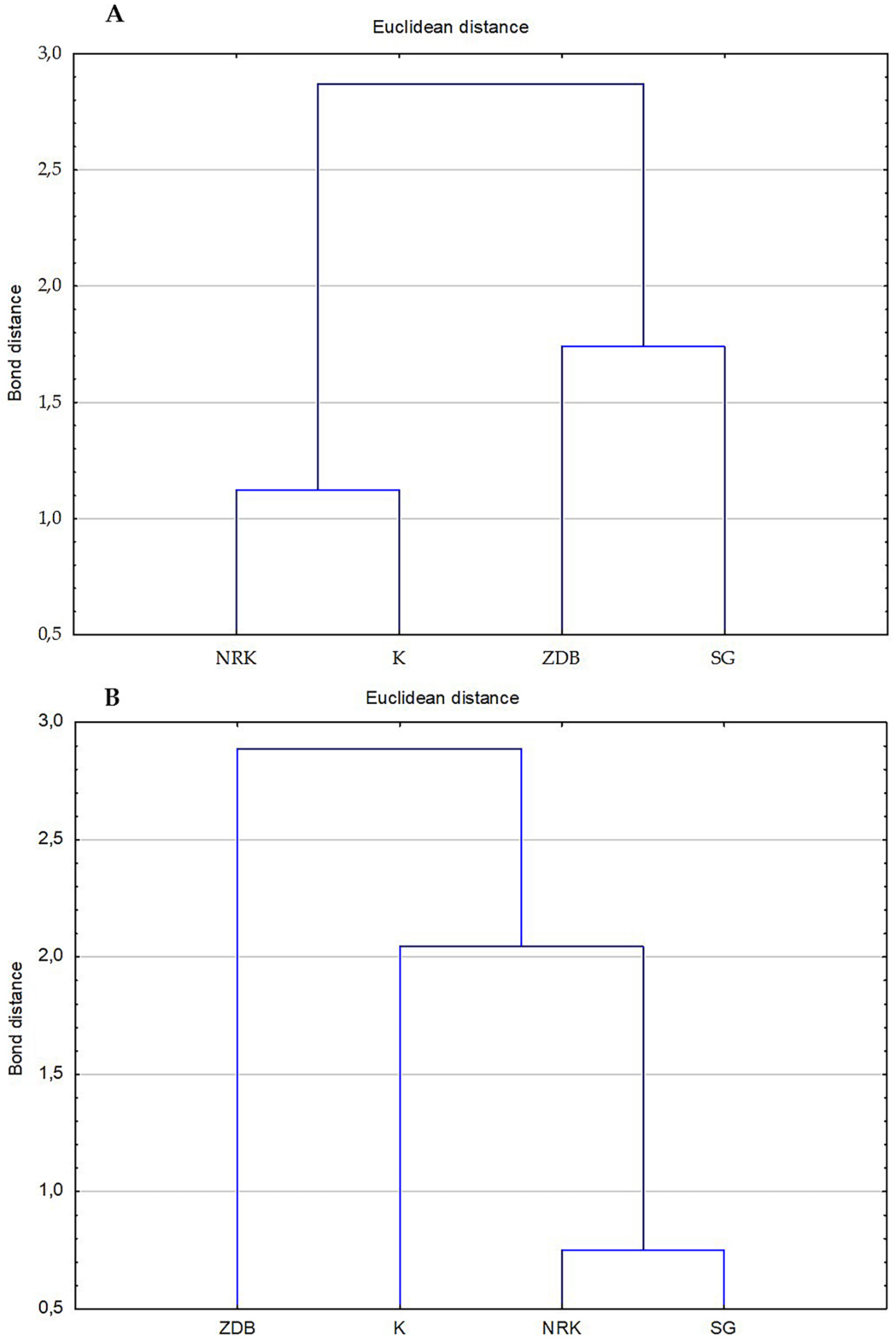

Figure 2. Ward's dendrogram for the overall enzymatic activity of (A) ungrazed areas and (B) grazed areas. SG - PLH060018 Stawska Góra, ZDB - PLH060035 Zachodniowołyńska Dolina Bugu, K - PLH060010 Kąty, NRK - Nature Reserve "Kózki"

objects. In the studied soils of the grazed plots, the effect of pH could be masked by the content of organic matter, which is a factor that has a greater impact on DhA.

Animal excrement is considered a valuable source of phosphorus in the soil environment (Rothwell et al., 2020). This is confirmed by the research of many authors, e.g., Liu et al. (2023) and Song et al. (2023), who also observed that grazing has a significant effect on the increase in the content of this element in the soil. On the other hand, Li et al. (2018) demonstrated the negative effects of grazing on organic matter content and phosphorus mineralization in the soil. The processes and mechanisms of phosphorus accumulation and its release and leaching from the soil are complex. They depend on factors such as pH, organic matter content, Fe and Al in the soil (Johan et al., 2021) and the activity of enzymes which catalyze organic phosphorus combinations

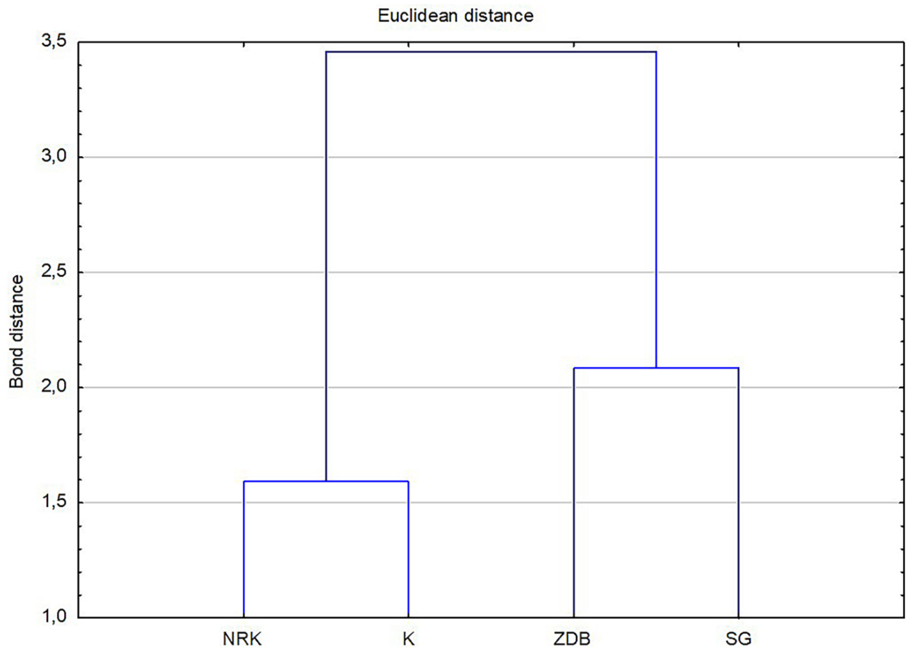

Figure 3. Ward's dendrogram for soil grain size. Explanations as in Figure 2

(Nannipieri et al., 2011; Liu et al., 2023). SEA from the phosphatase group is used to assess the potential rate of mineralization of these compounds in the soil (Bünemann, 2015). The intensity of phosphatases secretion by plants and microorganisms is strictly dependent on the plants' demand for phosphorus. The lower Pav content in autumn shown in these studies could be related to the intensive uptake of these nutrients by plants during the growing season. Significant nutritional needs of plants and the supply of fresh organic matter in the form of animal excrement stimulate the activity of phosphomonoesterases in the soil in autumn (Futa et al., 2016).

The activity of enzymes and physicochemical properties of the studied soils were very diverse in individual habitats, which was related to soil grain size. This was confirmed by a cluster analysis (Fig. 2). The soil from the Nature Reserve "Kózki" significantly differed in the level of biological activity from the soils of the other analyzed objects (Table 6–7) and grain size (Table 1). According to Lasota et al. (2021), soil grain size has a significant impact on the formation of soil biological activity. Soils containing more colloids generally provide better living conditions for plants and microorganisms, and their microbial activity is higher (Lasota et al., 2021). DhA is an integral part of living, intact microbial cells; thus, it better reflects the overall activity of microorganisms living in the soil. The cluster analysis also showed that sheep grazing had a significant impact on the formation of SEA.

Particularly noteworthy is the habitat of dry sand grasslands in the Nature Reserve "Kózki", where grazing of świniarki sheep was continued since 2008. As a result of nearly ten years of active protection of this habitat, a statistically significant increase in the value of the studied physicochemical and biochemical parameters was observed in the soil of the grazed plot compared to the control plot. In the soil of the grazed plot, about ten times higher activity of dehydrogenases and twice as high activity of neutral phosphatase were recorded compared to the control object. This confirms the hypothesis that free grazing of livestock has a positive impact on the biodiversity of the soil environment of valuable natural areas.

CONCLUSIONS

The obtained results indicate that biological activation and improvement of the chemical status of soils where sheep grazing takes place indicate a beneficial impact of this form of protection on the habitat and its biodiversity, as well as soil fertility and health. A particularly visible improvement in the biochemical condition of the soil was observed in the plot grazed by sheep since 2008. Statistical analysis of the results showed close positive correlations between SEA and the TOC and Pav content in the tested soils. This proves that an inflow of biogenic substances, also in the form of farm animal excrement, significantly stimulates the activity of soil enzymes. The demonstrated stimulation of SEA in sheep grazing conditions proves that this form of active protection of biodiversity of valuable natural habitats is effective and can contribute to the promotion of sheep breeding of native breeds.

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