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QUANTIFICATION OF BIODIVERSITY RELATED TO THE ACTIVE PROTECTION OF GRASSLAND HABITATS IN THE EASTERN LUBLIN REGION OF POLAND BASED ON THE ACTIVITY OF SOIL ENZYMES

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Abstract. This study focused on the impact of extensive sheep grazing on soil enzymatic activity in the grassland habitats of the eastern Lublin region of Poland, situated within the ecological Natura 2000 network of: Kąty (PLH060010), Stawska Góra (PLH060018), and Zachodniowołyńska Dolina Bugu (West Volhynian Valley of the Bug) (PLH060035). This study involved soils under sheep grazing and soils in uncultivated areas (wasteland). Two-year study was conducted in 2 periods of each year: spring (before the start of sheep grazing) and autumn (after grazing). Beneficial effects were found for extensive grazing by sheep on soil enzymatic activity within each tested habitat. It is worth underlining that a clear stimulation of enzyme activity was detected already in the first year of observation. In the second year, the enzyme activity in the soils of habitats under sheep grazing was approximately 1.5 times higher than in the soils without grazing. The observed higher activity of the tested enzymes in soils under sheep grazing indicated the usefulness of studies on the enzymatic activity of soils as a sensitive indicator of soil response, such as observed here with the applied active system of biodiversity protection in the valuable natural grassland habitats of Natura 2000.

Keywords: soils, enzymatic activity, Natura 2000, active protection, biodiversity, sheep grazing

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INTRODUCTION

Ensuring the stability of basic natural processes and the development of spatial interrelations between the different elements of the biosphere guarantees effective conservation of biodiversity resources and benefits in Natura 2000 areas (Gruszecki *et al.* 2011). The introduction of extensive sheep grazing to habitats to provide major biodiversity growth, such as the grassland habitats of the eastern territory of Lublin region (Kąty PLH060010, Stawska Góra PLH060018, Zachodniowołyńska Dolina Bugu PLH060035) covered by the BIOSTRATEG project (BIOSTRATEG2/297267/14/NCBR/2016), can improve their protection and the lasting use of biological and landscape diversity. The return to extensive grazing of farm animals enables the optimising of both commercial and protective actions (Futa *et al.* 2016). It is, therefore, a form of sustainable protection for grassland habitats in Natura 2000 areas.

Soil plays a major role in maintaining landscape biodiversity. It forms an essential component of habitats, determining the growth capacity of flora. The nature of the habitats and their natural plant systems is shaped by the soil properties and climate conditions present in the given area. Soil quality, in turn, is primarily shaped by the transformation of organic matter, mainly related to microorganisms and the enzymes they secrete (Nannipieri *et al*. 2002, Siwik-Ziomek and Lemanowicz 2016). Enzyme activity is considered to be one of the most sensitive indicators of ecosystem functioning. It reflects both the direction and the nature of the biogeochemical cycles, and all the fundamental transformations related to biology and the physico-chemical properties of soils (Russel *et al*. 2006, Bastida *et al.* 2008). Surveys of enzymatic activity provide early evidence of subtle changes in the soil environment, long before changes are observed in the chemical composition or physical properties of the soils (Bielińska *et al*. 2014).

The strategic goal of the present study is to identify the directions and dynamics of the changes occurring in soils of grassland ecosystems under the effect of extensive sheep grazing. This will facilitate the optimum selection of tools for further actions to protect the grassland habitat biodiversity within the Natura 2000 ecologic network, including adaptive management based on ecosystem evolution capacity. The utilitarian goal is to use enzymatic tests for the rapid evaluation of soil quality in these areas, and to predict further changes in the habitats by determining the effects of conservation operations on the stimulation of the self-regulatory processes in Natura 2000 grassland habitats.

MATERIALS AND METHODS

The study covers grassland habitats in the eastern Lublin region, located within the Natura 2000 ecology network: Kąty (PLH060010), Stawska Góra (PLH060018), Zachodniowołyńska Dolina Bugu (PLH060035). These habitats are located in varied physiographic units (3 mesoregions) belonging to the Lublin Upland and the Volhynian Upland (Kondracki 2002). They are characterised by rich relief and the presence of loess soils and chalk rendzina soils rich in calcium carbonate (Turski *et al.* 1993), specific rendzina created from carbonate rocks – Kąty and Stawska Góra, and typical lessivé soil formed from loess – Gródek (Zachodniowołyńska Dolina Bugu) (*Guide*… 2017). The object of the study included soils from areas subject to sheep grazing and from unused wastelands. The grazing of sheep on the habitats in question began in the spring of 2015. The study results presented in this paper cover the first two years of observations conducted as part of the BIOSTRATEG project (BIOSTRATEG2/297267/14/NCBR/2016).

Soil samples for laboratory analysis were taken from the 0–25 cm layer, on two separate days each year: in the spring (before the start of sheep grazing) and in the autumn (after the end of grazing), from test areas selected within the individual habitats. The soil sampling involved calculating the average of five samples taken from each area. The activities of the following enzymes: dehydrogenases (Thalmann 1968), acid phosphatase, alkaline phosphatase (Tabatabai and Bremner 1969), urease (Zantua and Bremner 1975) and pH in 1 mol·dcm^{-3} KCl (ISO 10390), organic carbon (ISO 14235), total nitrogen (ISO 13878) were assayed in the soil samples. All the determinations were made in three parallel replications.

RESULTS AND DISCUSSION

Beneficial effects of extensive sheep grazing on the enzymatic activity of the soils were observed in all the habitats (Tables 1–3).

TABLE 1. DEHYDROGENASES ACTIVITY (IN MG TPF·KG^{-1}·D^{-1})

Site	Year	Plot			
		Sheep grazing		Wasteland	
		Spring	Autumn	Spring	Autumn
Kąty PLH060010	2015	8.85	10.79	8.52	8.95
	2016	7.69	11.44	7.76	8.32
	$\bar{x}$	8.27	11.11	8.14	8.63
Stawska Góra PLH060018	2015	11.88	14.82	12.67	12.98
	2016	11.02	16.51	12.34	13.16
	$\bar{x}$	11.45	15.66	12.50	13.07
Gródek PLH060035	2015	17.59	23.57	17.98	18.49
	2016	16.12	23.98	17.15	17.84
	$\bar{x}$	16.85	23.77	17.56	18.16

It is worth stressing that the visible stimulation of the enzyme activity began during the first year of observations. In the second year, the enzyme activity in the soils of the habitats used for sheep grazing was approximately 1.5 times greater than in the soils from areas not subject to grazing (Tables 1–3). Sheep grazing is a stimulator of pasture sward, e.g. as a result of selective picking, treading of the ground and turf, and leaving of droppings (Mroczkowski 2011). The balanced development of flora affects the accumulation of specific substrates for enzymatic reactions in the soil, which markedly stimulates the activity of enzymes in the soil environment (Bielińska and Mocek-Płóciniak 2009, Bielińska *et al.* 2014). Enzyme activity depends on their absolute amount, the size of the pool of reactive compounds other than enzymes, and on catalytic efficiency. The soil environment adds further factors (abiotic and biotic) affecting catalytic efficiency, such as: content of mineral and organic colloids, temperature, water and air properties, pH, and the quantity and species of microorganisms. These factors are to a significant extent shaped by the applied protection system and the permanent use of habitat biodiversity. The beneficial effect of sheep grazing on the biological condition of soils has also been demonstrated in the "Kózki" nature preserve, located in the "Podlaski Przełom Bugu" landscape park, within the Natura 2000 areas (Bielińska and Gruszecki 2011, Gruszecki *et al.* 2011). Previous studies by Bielińska and Gruszecki (2010) demonstrated that the lack of use of the sward in the "Kózki" nature preserve was the result of secondary succession in sand-based sward communities, manifesting in reduced biodiversity and detrimental changes to the ecochemical condition of the soils.

TABLE 2. PHOSPHATASES ACTIVITY (IN MG PNP·KG^{-1}·H^{-1})

Site	Year	Plot			
		Sheep grazing		Wasteland	
		Spring	Autumn	Spring	Autumn
Kąty PLH060010	2015	8.63	11.39	8.94	9.12
	2016	7.11	10.73	8.27	8.65
	$\bar{x}$	7.87	11.06	8.60	8.88
Stawska Góra PLH060018	2015	12.56	17.33	13.15	13.46
	2016	10.22	15.21	11.38	11.59
	$\bar{x}$	11.39	16.27	12.26	12.52
Gródek PLH060035	2015	15.85	22.51	16.46	16.91
	2016	12.86	19.54	15.23	15.89
	$\bar{x}$	14.35	21.02	15.84	16.40

Soils in individual habitats differed significantly in the levels of enzymatic activity (Tables 1–3). This was probably related to local differences in soil properties, resulting from their different genesis, as well as the type of flora. Each soil type is characterised by both a particular composition of specific enzymes and its own enzymatic activity level. The differences in the shaping of the enzyme activities in different soils are mainly caused by the fact that each soil type, depending on its origin and its development conditions, is different in terms of organic matter content, granulometric composition and microorganism activity (Bielińska and Mocek-Płóciniak 2009). Another possible factor to modify the activity of the enzymes is the diverse species composition of the flora, which affects the accumulation of specific substrates for the enzymatic reactions in soils (Bielińska and Gruszecki 2010). The effect of higher plants on the soil enzymes depends on the chemical composition of the plant, which even in the case of root secretions may differ between different genera, species and variety. The individual influences of specific plant species on the enzymatic activity of soils is linked with the different species compositions of the bacteria colonizing the plant roots. Plants can affect enzyme activity directly by increasing their absolute amount and indirectly by changes in the content of organic matter and microorganism population (Bielińska *et al.* 2014).

TABLE 3. UREASE ACTIVITY (IN MG $N\text{-}NH_4^+ \cdot KG^{-1} \cdot H^{-1}$)

Site	Year	Plot			
		Sheep grazing		Wasteland	
		Spring	Autumn	Spring	Autumn
Kąty PLH060010	2015	13.32	18.24	14.16	15.52
	2016	12.98	19.73	14.35	16.02
	$\bar{x}$	13.15	18.98	14.25	15.77
Stawska Góra PLH060018	2015	16.19	22.74	17.51	18.48
	2016	15.67	23.82	16.29	17.67
	$\bar{x}$	15.93	23.28	16.90	18.07
Gródek PLH060035	2015	18.41	25.65	19.28	20.16
	2016	17.24	26.38	18.56	19.40
	$\bar{x}$	17.82	26.01	18.92	19.78

The highest enzyme activity was noted in the soil from the Gródek habitat PLH060035 (Tables 1–3). The observed stimulation of enzymatic activity was related to the total organic carbon and nitrogen contents (Table 4), much higher than in the soils from the other habitats (Kąty PLH060010 and Stawska Góra PLH060018). The literature data (Aon and Colaneri 2001, Kieliszewska-Rokicka 2001, Domżał and Bielińska 2007) often indicate that the activity level of soil enzymes is closely related to the content of organic matter in the soil.

TABLE 4. CONTENT OF ORGANIC CARBON (C_{ORG}), TOTAL NITROGEN (N_T), RATIO C:N AND PH_{KCL}

Site	Year	Term	Sheep grazing C_{org} [g·kg^{-1}]	Sheep grazing N_t [g·kg^{-1}]	Sheep grazing C:N	Sheep grazing pH KCl	Wasteland C_{org} [g·kg^{-1}]	Wasteland N_t [g·kg^{-1}]	Wasteland C:N	Wasteland pH KCl
Kąty PLH060010	2015	Spring	25.22	2.54	9.9	7.3	25.85	2.48	10.4	7.4
		Autumn	24.80	2.42	10.2	7.4	22.44	2.19	10.2	7.3
	2016	Spring	25.59	2.48	10.3	7.2	24.16	2.34	10.3	7.2
		Autumn	26.02	2.58	10.0	7.3	20.48	1.96	10.4	7.3
Stawska Góra PLH060018	2015	Spring	32.61	3.23	10.1	7.0	33.54	3.21	10.4	7.0
		Autumn	32.26	3.22	10.0	7.1	32.08	3.11	10.3	7.1
	2016	Spring	32.48	3.21	10.1	7.0	34.80	3.34	10.4	7.0
		Autumn	32.11	3.08	10.4	7.1	32.92	3.20	10.2	7.1
Gródek PLH060035	2015	Spring	41.78	4.25	9.8	7.2	42.24	4.26	9.9	6.9
		Autumn	40.94	4.07	10.0	7.1	41.06	4.17	9.8	7.0
	2016	Spring	42.38	4.34	9.7	7.2	45.12	4.58	9.8	6.9
		Autumn	39.26	4.02	9.8	7.3	44.61	4.49	9.9	7.1

The data in Table 4 indicate that during the short two-year observation period there were no visible effects of sheep grazing on the chemical properties of the soils. The minor fluctuations in total organic carbon and nitrogen content may have been related to meteorological conditions. It is known that elevated air temperatures and overdrying of soils increase the accumulation of organic matter, while wet weather and reduced temperature decrease it. The soils in an ecosystem depend on soil-forming factor systems, which have different effects over time, and which are diverse and variable in space. All the ecological, sozological, resource and functional, self-regulating and immunising, and buffering structures of ecosystems are interconnected. They form complex systems of processes and phenomena, for which comprehensive research, design and formation are unusually difficult. The objectivism and complete characterisation of the processes occurring in the soil environment require long-term monitoring (Gruszecki *et al.* 2011). The assessment of soil quality is not simple due to the complexity of the soil environment and the variability of the conditions present therein, especially those related to the buffer capacities of a sorption complex and to the retention and accumulation of compounds in the soil. Clear changes in the chemical properties of soils are shaped by the long-term effects of the same natural and anthropogenic factors. This especially applies to calcium-based soils. Soils originating from carbonate rocks form stable organic-mineral complexes, whereas the mineralisation processes are very slow. Humus usually takes the form of a calcimorphic mull or calcimorphic mull-moder (Mocek and Drzymała 2010). Futa *et al.* (2016) demonstrated changes in the chemical properties of soils under the effects of extensive farm animal grazing after 3–4 years of grazing.

CONCLUSIONS

1. The introduction of extensive sheep grazing in the grassland habitats (Kąty PLH060010, Stawska Góra PLH060018, Zachodniowołyńska Dolina Bugu PLH060035) fosters the greater biological activity of the soils.

2. The reactions of the enzymes, expressed as a significant leap in their activities in areas subject to sheep grazing, indicate the usefulness of testing the enzymatic activity of soils as a sensitive indicator of soil reaction to the applied active protection system for the biodiversity of naturally-valuable Natura 2000 grassland habitats.

3. In considering the ecological aspect, it is important that the high enzymatic activity of soils in sheep grazing areas was observed during a two consecutive year period, which may indicate that this soil condition has become more stable.

4. The planned long-term monitoring of changes to the pedosphere within the surveyed grassland habitats will make possible the assessment of the effects of farm animal grazing on the chemical properties of soils. The observations performed so far indicate that the chemical properties of the surveyed soils are mainly shaped by internal factors.

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