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BARBARA FUTA\*, KRZYSZTOF PATKOWSKI\*\*,  
ELŻBIETA J. BIELIŃSKA\*, TOMASZ M. GRUSZECKI\*\*,  
MICHAŁ PLUTA\*\*\*, MARIUSZ KULIK\*\*\*\*, SZYMON CHMIELEWSKI\*

## SHEEP AND HORSE GRAZING IN A LARGE-SCALE PROTECTION AREA AND ITS POSITIVE IMPACT ON CHEMICAL AND BIOLOGICAL SOIL PROPERTIES

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*Abstract.* This paper looks into the impact of free grazing by sheep and horses on the chemical and biological properties of soils in the partial protection zone of the Roztocze National Park. The study sampled three different types of pastureland in the area: pastures for sheep, horses and for combined grazing by sheep and horses. Compared to an ungrazed reference pasture, free grazing significantly stimulated the activity of enzymes which catalyze the transformation of organic matter (dehydrogenases, phosphatases and ureases) and it also positively influenced other chemical properties of the soils. Among the soils under free-grazing management, the pasture grazed to horses underwent the most advantageous changes in terms of the eco-chemical status of the soil.

**Keywords:** free grazing, large-space protected area, soil, chemical properties, enzymatic activity

### INTRODUCTION

One of the major conservation-related tasks in Poland, stemming from the Habitats Council Directive of the EEC (1992), is to ensure the proper condition of large-space protected areas such as the Roztocze National Park (RNP). One

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\* Institute of Soil Science, Environment Engineering and Management.

\*\* Department of Small Ruminant Breeding and Agricultural Advisory.

\*\*\* Department of Horse Breeding and Use.

\*\*\*\* Chair of Grassland and Landscape Formation,  
University of Life Sciences, Lublin, Poland, 20-950 Lublin, Akademicka 13  
Corresponding author's e-mail: [barbara.futa@up.lublin.pl](mailto:barbara.futa@up.lublin.pl).

important goal is to maintain the characteristic features of the landscape inherent to the region (Chmielewski *et al.* 2014). In Poland, long-term surveillance of the changes to the flora and fauna in areas under conservation has been conducted. However, changes to the pedosphere have been looked into only at snapshot intervals (Konecka-Betley *et al.* 2002; Bielińska and Gruszecki 2010).

The conservationist approach to nature protection, where protected areas are insulated to a larger or smaller extent from human interference, is being replaced by an active shaping of the landscape based on the premises of the European Landscape Convention (ELC 2000). Among the various approaches to active nature conservation, free grazing may have a beneficial effect on the biological status of soils and on the biodiversity of protected areas including valuable plant communities (Bielińska and Gruszecki 2011; Gruszecki *et al.* 2011; Mroczkowski 2011).

Forecasting changes in the environment due to the practices of active habitat protection is a tough proposition as these changes oftentimes elude straightforward predictions (Chmielewski 2006). Wätzold (2006) proposed the use of measurable indicators which would make it possible to quantify the environmental effects of the implementation of proposed protection measures. To this end, communicative enzyme-based indicators have been suggested as indicators because they simultaneously provide information about the current eco-chemical status of soils as well as changes to a habitat. As such communicative enzymes may be used to quantify the environmental effects of active protection applied to habitats of natural value (Bielińska and Gruszecki 2011). Ecosystem evaluation based on enzymatic tests does not merely offer the possibility to run a series of analyses but, furthermore, provides an opportunity to summarize the effects of numerous factors and to evaluate unique parameters such as cellular metabolites (Nortcliff 2002; Maurel and Ricard 2006; Bielińska *et al.* 2008; Bielińska *et al.* 2014; Oleszczuk *et al.* 2014a, 2014b).

This study was designed to evaluate the effect of free grazing by sheep and horses on the chemical properties of soils within the confine of the partial protection zone of the RNP.

## MATERIALS AND METHODS

The study was conducted during 2013–2014 in a village named Zwierzyniec within the RNP, specifically in the mid-forest settlement of Florianka (22°58'56 E, 50°33'10 N). In terms of the physiogeographic division, the RNP is situated close to the eastern border of the Western Europe territory in the province of Małopolska (34), the central part of the macro-region of Roztocze (343.2). It occupies the southeastern slope of the mezo-region of Western Roztocze (343.21) and the northwestern slope of the mezo-region of Central Roztocze (343.22) (Kondracki 2002). In biogeographic terms, the RNP is situated in the Continental Region of

Europe (European Environment Agency 2007). The RNP's landscape is formed by rolling hills which provide the division between the river basins of the Dniester and the Vistula. Hydrogeographically, the RNP belongs to the catchment area of the Wieprz, the right-side tributary of the Vistula.

The prevailing part of the natural RNP's landscape consists of insulated cretaceous plateaus and strongly dissected loess uplands. They are complemented by river floodplains and, locally, by marshy lowlands. Twelve types of soils have been identified within the RNP, with rendzina and podsollic soils accounting for the largest area (33% each) followed by rusty soils (24%). In terms of land use, 95.5% of the national park is forested. Farmland (meadows and arable land) accounts for approximately 3.1% of the park with water covering around 0.6% of the total area. The forests are dominated by stands of pine (55.8%) fir (19.4%) and beech (17.4%) (Reszel and Grądziel 2013).

There are 924 species of vascular plants in the RNP alongside 237 species of bryophytes and 230 species of lichens. The RNP is also rich in valuable fauna; the most emblematic of which is a horse, the Polish primitive horse, an alleged descendant of the extinct tarpan species *Equus gmelini* (Reszel and Grądziel 2013).

The areas from which soil samples were collected in the study were four pastures, three of which were used for grazing (Figure 1):

- sheep grazing – 0.7 ha (area No. 1);
- sheep and horse grazing – 1.0 ha (area No. 2);
- horse grazing – 0.7 ha (area No. 3).

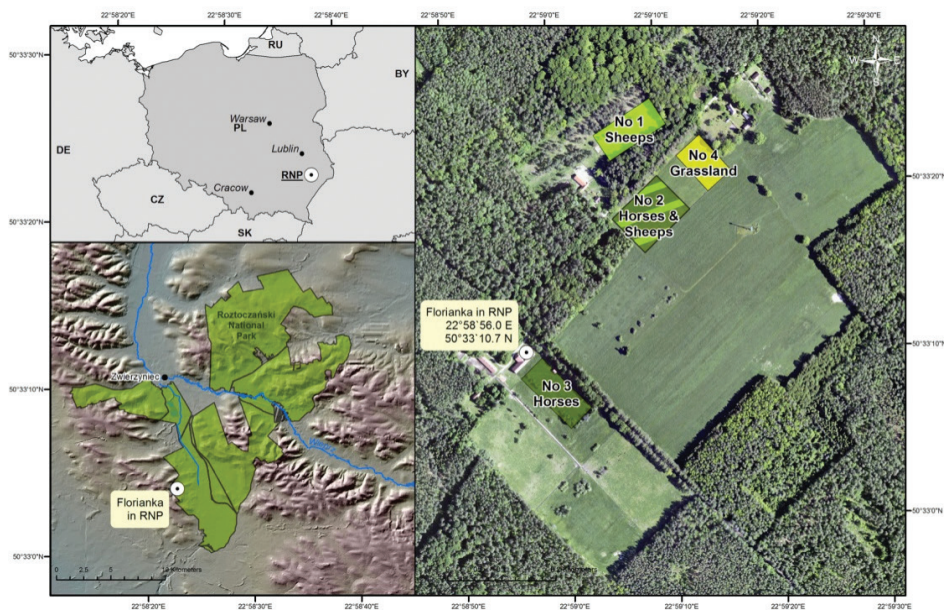


Figure 1. Florianka study area, located in the RNP (East Poland).

The above pastures have been operational since 2010. In terms of livestock units (LU), their stocking rate was 8.0 LU for sheep, 3.7 LU for horses, and 9.2 LU for combined grazing by sheep and horses. A grassland used only for hay, 0.5 ha (area No. 4), that was in close vicinity to the grazed pastures yet outside of the grazing area provided the reference soil samples.

The area under study was comprised of soils formed from eolian and fluvioglacial sands which have the granulometric composition of loose sand. The vegetation included *Molinio-Arrhenatheeretea* and *Koelerio glaucae-Corynephoretea canescentis* plant assemblages interspersed with *Juniperus communis* and *Pinus silvestris* growing singly or in small clusters.

Each year soil was sampled for the study on two dates: before the grazing season (April) and on its termination (October) during periods of stable weather when the soil was in a state of dynamic equilibrium with biochemical processes running at moderate rates. The soil was sampled at 5 different points on each surface. Each sample was individually averaged within the range of studied areas and was assayed in triplicate. The samples were collected from the humus horizon at a depth of 0–25 cm.

Laboratory analyses were performed to determine the activities of the following enzymes: dehydrogenases (Thalman, 1968), phosphatases (Tabatabai and Bremner 1969), urease (Zantua and Bremner 1975), pH in 1 mol·dm<sup>-3</sup> KCl (ISO 10390); organic carbon (ISO 14235), total nitrogen (ISO 13878), ammonia and nitrate nitrogen (ISO 14255) as well as available forms of phosphorus (ISO 11263). The total content of Zn, Pb, Mn and Cu was determined with emission spectrometry using a Leeman Labs (PS 950) apparatus with ICP induction in argon. Soil samples were mineralized in a PROLABO microwave oven (Microdigest 3.6, France) with a wet method, which uses a mixture of nitric acid and perchloric acid in a 1:1 ratio (Baran *et al.* 2002). Statistical analyses were performed with PCA tests.

Weather conditions prevailing during the study period (precipitation and monthly air temperature averages) are shown in Table 1. The data were obtained from the Roztocze National Park Base Station.

TABLE 1. METEOROLOGICAL DATA IN 2013–2014 IN SB RPN

Years	Month							
	IV	V	VI	VII	VIII	IX	X	IV–X
Monthly precipitation sums (mm)								
2013	54.8	153.2	125.2	44.5	6.6	63.5	7.5	455.3
2014	35.0	87.6	49.6	111.8	97.6	47.4	40.9	469.9
Mean of many temperature (°C)								
2013	7.4	15.0	17.8	18.5	18.4	11.2	9.6	13.9
2014	9.3	13.2	15.7	20.0	17.5	13.9	9.0	14.1

## RESULTS AND DISCUSSION

Pasture soils showed a higher pH than did the reference soils that were outside the grazing area. The pH values (in 1 mol·dm<sup>-3</sup> KCl) ranged from 0.14–1.40 in the spring to 0.46–1.82 in the autumn (Table 2). In the period covered by the study, the greatest pH<sub>KCl</sub> values were found in the soil from the pasture grazed to horses. The increase in pasture soil pH<sub>KCl</sub> could be related to the microbiological decomposition of uric acid excreted by animals (Bielnińska and Gruszecki 2011). Ammonia is the primary end product of the decomposition which enters the soil solution and forms NH<sub>4</sub>OH. Ammonium ions have been shown to be a major soil-alkalizing factor (Gay and Knowlton 2005). In addition, animal droppings themselves are rich in alkaline elements (Abrahams and Steigmajer 2003). Within the pasture area, there was a tendency for the soil pH<sub>KCl</sub> to increase over time (Table 2).

TABLE 2. PH, ORGANIC CARBON AND TOTAL NITROGEN CONTENTS, C:N RATIO

Plot	Years	Seasons	pH <sub>KCl</sub>	C (g·kg <sup>-1</sup> )	N (g·kg <sup>-1</sup> )	C:N
sheep pasturage	2013	Spring	6.42	11.49	1.09	10.5
		Autumn	6.17	12.38	1.16	10.6
	2014	Spring	7.17	13.61	1.32	10.3
		Autumn	6.61	12.97	1.23	10.5
horses pasturage	2013	Spring	7.21	16.53	1.59	10.4
		Autumn	6.96	18.85	1.78	10.6
	2014	Spring	7.35	19.20	1.81	10.6
		Autumn	7.19	17.76	1.71	10.3
horses and sheep pasturage	2013	Spring	6.81	14.44	1.38	10.4
		Autumn	6.56	17.62	1.66	10.6
	2014	Spring	7.12	17.32	1.63	10.6
		Autumn	7.05	15.83	1.49	10.6
meadow	2013	Spring	6.28	10.87	1.04	10.4
		Autumn	5.71	11.54	1.14	10.1
	2014	Spring	5.95	11.48	1.11	10.3
		Autumn	5.57	10.95	1.08	10.1
LSD <sub>0.05</sub> for: Plot				4.28	0.43	n.s.
Years				3.46	0.32	n.s.
Seasons				3.46	0.32	n.s.

Free grazing had a beneficial effect on the content of organic carbon and total nitrogen of the soils. The effect was particularly manifest for the pasture grazed to horses where the contents of organic carbon and total nitrogen were about 1.5-fold higher than those found in the reference soil (Table 2). These results reflect the influx of fresh organic matter to the soil environment via ani-

mal droppings. Viewed from a season-to-season perspective (based on data in Table 1), the pastures responded to increased temperatures and to drying out by more rapidly increasing in organic matter, whereas abundant rainfall and reduced temperatures resulted in a slower rate of organic matter accumulation. There was no evident impact of grazing in terms of organic carbon or total nitrogen contents over the years of the study, although within pastures there was a tendency for those soil constituents to increase (Table 2). The ratio of carbon to nitrogen in the soils did not vary during the study period and stayed within a range of 10.1 to 10.6 (Table 2).

The pasture soils had a higher mineral nitrogen contents ( $\text{N-NH}_4^+$  and  $\text{N-NO}_3^-$ ) than did the reference soil but in the majority of cases the differences were not significant (Table 3). In the grazed soils, uric acid excreted with droppings is the major source of heterogenous nitrogen (Gaines and Gaines 1994; Gay and Knowlton 2005; Ligęza 2009; Bielińska and Mocek-Płóćiniak 2015). Both pasture and reference soils displayed high  $\text{N-NO}_3^-$  contents (Table 3). Nitrification, the outcome of enhanced mineralization, is reported to be the major cause behind the rise of nitrate nitrogen in the environment (De Boer *et al.* 1990; Bielińska 2006). With an increased influx of nitrogen, immobilization of nitrogen in soil microorganisms declines, whereas nitrogen mineralization increases (Tietema and Van Dam 1996; Bielińska 2006). In the soils under study, the content of nitrate was approximately 10 times higher than ammonium. Relative levels of nitrate and ammonium ions in soil are significantly influenced by pH. The slightly acidic or neutral pH of the soils (Table 2) may have contributed to an increased rate of microbiological oxidation of the nitrate. The nitrogen contents were also higher in the spring than in the autumn, which may reflect the uptake of the compounds by plants or their leaching from topsoil by rainfall.

The soils under pasture had more available phosphorus than did the reference soil. In the period covered by the study, the horse pasture was found to have the highest phosphorus content, approximately 2–3 times higher than that of the remaining grazed areas. Phosphorus levels correlated to the volume of fresh organic matter supplied to the soil via animal droppings (Table 3). Similar to what was observed with nitrogen, phosphorus was lower in the autumn than in the spring (Table 3), a phenomenon likely caused by intensive uptake of phosphorus by plants in the spring.

Soils under grazing also tested higher for heavy metals than the reference soil, but the difference was statistically significant in the horse pasture (Table 4). Heavy metals may be added to pasture soils directly by animal defecation and urination (Abrahams and Steigmajer 2003; Wilkinson *et al.* 2003; Ajorlo *et al.* 2010). Judging from the current standards (Kabata-Pendias *et al.* 1993) the tested soils had inherently high contents of heavy metals.



TABLE 3. CONTENT OF NITROGEN (N-NH<sub>4</sub><sup>+</sup> AND N-NO<sub>3</sub><sup>-</sup>) AND AVAILABLE PHOSPHORUS FORMS (P)

Plot	Years	Seasons	N-NH <sub>4</sub> <sup>+</sup>	N-NO <sub>3</sub> <sup>-</sup>	P
			(mg·kg <sup>-1</sup> )		
sheep pasturage	2013	Spring	15.54	148.54	101.89
		Autumn	14.53	135.71	78.78
	2014	Spring	13.29	126.50	101.08
		Autumn	12.18	119.63	63.68
horses pasturage	2013	Spring	22.69	185.40	298.45
		Autumn	16.51	149.59	283.69
	2014	Spring	20.30	155.38	306.38
		Autumn	13.42	131.44	290.61
horses and sheep pasturage	2013	Spring	18.63	154.82	121.49
		Autumn	13.36	134.96	86.92
	2014	Spring	18.23	151.16	114.56
		Autumn	17.94	129.39	70.41
meadow	2013	Spring	14.68	134.75	96.84
		Autumn	12.96	122.17	46.31
	2014	Spring	14.58	123.88	61.22
		Autumn	12.48	119.73	34.09
LSD <sub>0.05</sub> for: Plot			5.52	34.63	22.78
Years			4.34	27.92	18.56
Seasons			4.34	27.92	18.56

TABLE 4. CONTENT OF TOTAL FORMS OF HEAVY METALS IN MG·KG<sup>-1</sup> DM OF SOIL

Plot	Years	Seasons	Zn	Pb	Mn	Cu
sheep pasturage	2013	Spring	14.6	9.8	121.1	1.2
		Autumn	11.9	8.9	106.4	1.0
	2014	Spring	9.1	8.2	109.0	1.1
		Autumn	8.7	7.8	100.3	0.9
horses pasturage	2013	Spring	16.6	12.9	128.0	2.5
		Autumn	14.9	13.4	108.8	1.3
	2014	Spring	13.1	14.5	116.2	3.1
		Autumn	12.6	10.8	104.7	1.9
horses and sheep pasturage	2013	Spring	15.2	11.6	114.3	1.6
		Autumn	12.1	10.5	100.9	1.2
	2014	Spring	8.9	11.1	126.0	2.2
		Autumn	7.3	9.9	104.1	1.2
meadow	2013	Spring	7.8	7.1	84.5	0.9
		Autumn	8.8	7.6	74.2	0.7
	2014	Spring	7.4	6.9	76.3	0.9
		Autumn	7.3	6.7	63.4	0.8
LSD <sub>0.05</sub> for: Plot			4.8	3.9	49.8	0.4
Years			4.2	3.4	42.1	0.3
Seasons			4.2	3.4	42.1	0.3

Free grazing significantly stimulated the enzymatic activity of the soils. The horse pasture soil tested the highest for enzyme activity, around 2–3 times higher than the reference soil (Table 5). Organic carbon and total nitrogen levels were parallel with enzyme activities being higher in the horse pasture soil than in the other soils. The enhanced enzymatic activity was likely also promoted by the beneficial changes in soil pH by horse grazing (Table 2). Furthermore, there was a close relationship between the activity of the enzymes and the soil contents of organic carbon and total nitrogen (Table 6).

TABLE 5. ENZYMATIC ACTIVITY OF SOILS

Plot	Years	Seasons	DhA	PhA	UA
sheep pasturage	2013	Spring	26.33	12.57	29.57
		Autumn	25.23	6.41	25.34
	2014	Spring	13.22	5.98	14.42
		Autumn	11.58	3.19	11.83
horses pasturage	2013	Spring	38.64	23.82	36.10
		Autumn	35.76	12.46	30.41
	2014	Spring	17.48	11.94	18.75
		Autumn	16.52	6.55	15.28
horses and sheep pasturage	2013	Spring	27.61	19.44	33.06
		Autumn	26.44	8.76	27.15
	2014	Spring	14.38	9.25	15.16
		Autumn	12.59	4.16	12.31
meadow	2013	Spring	16.34	9.32	11.80
		Autumn	15.12	3.85	10.21
	2014	Spring	6.67	3.96	5.19
		Autumn	5.04	1.98	4.53
LSD <sub>0.05</sub> for: Plot			3.38	1.33	2.98
	Years		2.66	1.12	2.32
	Seasons		2.66	1.12	2.32

DhA – dehydrogenases in  $\text{cm}^3 \text{H}_2 \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$

PhA – phosphatases in  $\text{mmol PNP} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$

UA – urease in  $\text{mg N-NH}_4^+ \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$

These findings corroborate previous reports from the literature (Aon and Colaneri 2001; Domżał and Bielińska 2007) which found the activity of soil enzymes is closely related to the content of organic matter. Likewise, other authors have reported the beneficial effect of increased soil pH on enzymatic activity (*inter alia* Acosta-Martinez and Tabatabai 2000; Zhao *et al.* 2009). Interestingly, the enzymatic activity was several times higher in 2013 than in 2014 in all pastures (Table 5). One reason behind the difference could be the lower total rainfall in April to June and in September of 2014 resulting in desiccation of soil. Seasonal variations in enzymatic activity are primarily related to the changes in soil aeration and moisture levels and are almost independent of



the small differences in soil carbon and nitrogen contents (Pascual *et al.* 2007; Bielińska *et al.* 2014). Studies of the seasonal changes in enzyme activity of soils have shown that the enzymatic activity is affected by numerous factors such as temperature, moisture, vegetation, short-term fluctuations in bacterial number and biomass, influx of fresh organic matter to the soil and leaching of enzymes. Furthermore, the development of vegetation cover can affect enzyme activity because it results in direct secretion of enzymes into the rhizosphere and in the development of microorganisms in the root zone (Bielińska *et al.* 2014). Seasonal changes in soil physicochemical properties can also cause major changes in the enzymatic activity of the soil, especially because of the longevity of enzymes immobilized in the soil.

TABLE 6. CORRELATION COEFFICIENTS BETWEEN THE ACTIVITY OF THE EXAMINED ENZYMES AND CONTENT OF ORGANIC CARBON (C), PHOSPHORUS (P) AND HEAVY METALS (N = 16).

Enzymes	C	N-NH <sub>4</sub> <sup>+</sup>	N-NO <sub>3</sub> <sup>-</sup>	P	Zn	Pb	Mn	Cu
Dehydrogenases	n.s.	0.526**	0.778**	0.558**	0.895***	0.674**	0.625**	n.s.
Phosphatases	n.s.	0.790**	0.944***	0.589**	0.841***	0.702**	0.678**	0.606**
Urease	n.s.	0.550**	0.789**	n.s.	0.928***	0.695**	0.958***	n.s.

\*\*\*significant at P<0.001

\*\*significant at P<0.01

n.s. – not significant

Other factors that significantly and positively correlated with increased enzyme activity were inorganic nitrogen levels (N-NH<sub>4</sub><sup>+</sup> and N-NO<sub>3</sub><sup>-</sup>), available phosphorus and total contents of heavy metals (Table 6). Addition of biogenic substances like these seems to substantially stimulate the activity of soil enzymes. The impact of heavy metals on the biological activity of soil, though, is dependent on their content in the environment. If they are present in the soil in amounts that approximate the natural values then the activity of soil enzymes may be stimulated. However, if they are present in excess, heavy metals can become enzyme inhibitors (Liao and Xie 2007).

## CONCLUSIONS

This study showed that the free grazing of sheep and horses had a beneficial effect on chemical and biological properties of soils within the partial protection zone of the RNP.

Among other benefits, free grazing enhanced the activity of enzymes involved in the transformation of organic matter in the soil. The essential envi-

ronment conservation-related aspect was that the enhancement of the biological activity of the soils under pasture occurred across four consecutive grazing seasons, thereby indicating the consolidation of the soil status. Of all the soils under grazing, the greatest enzymatic activity was found in the pasture grazed to horses. The increase was closely related to organic carbon levels and available phosphorus contents and was also linked to increased soil pH.

These beneficial changes, which underscore the overall eco-chemical status of the soils in the RNP area, support the idea that those soils require active protection to maintain their biodiversity. Our results further suggest that optimal protection is provided by introducing free grazing of sheep and horses to the pastureland in the RNP.

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