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ASSESSMENT OF SOIL BIOLOGICAL ACTIVITY UNDER CONDITIONS OF LONG-TERM DIVERSIFIED MINERAL FERTILIZATION

OCENA AKTYWNOŚCI BIOLOGICZNEJ GLEBY W WARUNKACH DŁUGOTRWAŁEGO ZRÓŻNICOWANEGO NAWOŻENIA MINERALNEGO

Abstract: Biological activity is investigated at different levels in cases of disturbances of processes, taking into consideration C and N changes in time and modern models of carbon sequestration. Soil properties created by fertilization are one of the factors influencing soil respiratory activity. This activity was measured by the manometric method in the conditions of long-term fertilizing experiment after 44 years management of the mountain meadow. One object chosen for the investigation was fertilized only with P and K and two objects were fertilized with ammonium nitrate in two doses: 90 and 180 kgN $\cdot$ ha^{-1} in the background of PK. The experiment was conducted with the limed soil and in series without liming. Manometric measurement comprised change of pressure in a closed container which was proportional to oxygen consumption and created by respiratory processes taking place in it. The equivalent amount of created CO_2 was absorbed by 1 mol $\cdot$ dm^{-3} NaOH solution. Biological activity of materials was expressed in mgO_2 $\cdot$ g^{-1} d.m. $\cdot$ d^{-1}. Equations of pressure changes indicate differences in soil respiration caused by fertilization intensification. Generally, systematic soil liming caused an increase in its biological activity. However, liming influenced this activity to a lower degree than mineral fertilization. The highest respiratory activity and dehydrogenase activity (μg TPF $\cdot$ g^{-1} f.m. $\cdot$ d^{-1}) was observed in the soil of the object fertilized with 90 kg N + PK $\cdot$ ha^{-1} and the object with fertilization increased P and K availability causing an increase in biological nitrogen fixation.

Keywords: biological activity, soil, long-term experiment

Introduction

It is considered [1, 2], that despite many factors (including moisture content or temperature) considerably affecting a temporary respiratory activity of soil, this

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indicator is a proper element describing the ecosystem metabolism. There is a strong relationship between soil respiration and the other processes occurring in it, including plant growth. In a review of the literature on the subject, Mocek-Plociniak [3] emphasizes a strong relationship between the activity of dehydrogenases and organic matter content, soil fertility, microorganism number, proteolytic activity, nitrification, denitrification, respiration, and the activities of other enzymes present in the soil environment. Generally many enzymes which belong to the dehydrogenase group produce anaerobic bacteria, therefore their intensified activity is observed under anaerobic conditions. Periodical changes of the enzyme activity are connected with changes in moisture content and soil aeration but do not depend on slight changes in C and N content in soil.

Modern models of carbon sequestration require improvement among others involving disturbances in carbon accumulation, dependencies in time of carbon and nitrogen transformations and their levels, or reactions on the border of biotic environment and substratum. On ploughlands long-term fertilization changes the soil properties and shapes plant communities. These conditions cause a change in the soil biological activity. Indirect methods of its assessment, respiration and dehydrogenase activity may be indicators of anthropopressure caused by management practices.

Material and methods

The investigations were conducted under laboratory conditions basing on the soil collected from the long-term fertilizer experiment in Czarny Potok. Soil samples from selected objects were collected in autumn 2011. The analyses were conducted in three objects (Table 1) selected from among 8 treatments managed in the experiment [4]. The object where only phosphorus and potassium fertilization was applied and objects fertilized with ammonium nitrate (90 and 180 kgN · ha^{-1} against PK) were chosen for the analyses. The experiment was conducted on the series with limed soil and without liming.

Table 1

Fertilization scheme in the static experiment in Czarny Potok

Fertilizer objects	Annual dose of the element in series 0 Ca and + Ca (1985, 1995, 2005) [kg · ha^{-1}]			Nitrogen fertilizer	Microelements
	P	K	N		
PK	39.24*	124.5*	—	—	B, Cu, Zn, Mn, Co, Mo
90 kg N + PK	39.24	124.5	90*	ammonium nitrate	B, Cu, Zn, Mn, Co, Mo
180 kg N + PK	39.24	124.5	180*	ammonium nitrate	B, Cu, Zn, Mn, Co, Mo

0 – Ca unlimed series; + – Ca limed series; * – doses of N, P and K fertilizers has been decreased by 1/3 since 2010.

A static fertilizer experiment [4], from which the soil samples were collected, has been conducted since 1968 in Czarny Potok near Krynica (20°54′53″ E; 49°24′35″ N). It

is situated at about 720 m a.s.l., at the foot of Jaworzyna Krynicka Mt, in the south-eastern Beskid Sądecki Mts, on a 7° land slope of NN exposure. The experiment was set up on a natural *Nardus stricta* L. and *Festuca rubra* type with a considerable proportion of the dicotyledonous. The soil from the experiment area was classified as brown acid soil developed from the Magura sandstone with granulometric composition of light silt loam (share of fractions: 1–0.1 mm – 40 %; 0.1–0.02 mm – 37 %; > 0.02 mm – 23 %) and characteristic three genetic horizons: AhA (0–20 cm), ABbr (21–46 cm) and BbrC (47–75 cm). The details of the experiment were presented in a previous paper [4] and in Fig. 1.

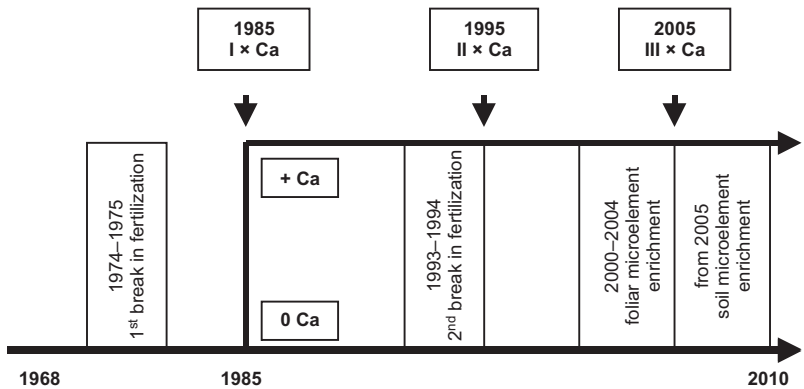

Fig. 1. Scheme of modification of cultivation measures in the experiment

Since autumn 1985 the experiment has been maintained in two series: limed and without liming, at the same level of NPK fertilization. In 1995 and 2005 liming was repeated. The lime doses for the first and third liming were calculated on the basis of 0.5 Hh value, whereas the second measure considered total hydrolytic acidity assessed in the year preceding the liming.

No mineral fertilization was applied in 1974–1975 and 1993–1994 and the investigations were limited to the assessment of the sward yield and its chemical composition.

In 1968–1980 phosphorus and potassium were sown in autumn. Since 1981 these fertilizers have been sown in spring, whereas potassium (1/2 dose) was supplemented in summer after the first cut. Thermal phosphate was used in 1968–1973, whereas triple superphosphate (46 %) has been applied since 1976 and enriched superphosphate (40 %) since 2005. Over the entire period of the experiment the nitrogen fertilizers were sown on two dates: 2/3 of the annual dose in spring when the vegetation started and 1/3 dose two weeks after the 1st cut harvesting. In 1994 10 kgCu and kgMg · ha^{-1} was applied once as regenerative fertilization. In 2000–2004 foliar fertilization was applied (2 dm^3 · ha^{-1} used twice) with Mikrovit-1 microelement fertilizer composed of 23.3 gMg; 2.3 gFe; 2.5 gCu; 2.7 gMn; 1.8 gZn; 0.15 gB and 0.1 gMo per 1 dm^3. In 2005–2007 0.5 kgB per 1 ha was applied to the soil annually, whereas in 2008 in spring 5 kgCu, Zn and Mn each per 1 ha and 0.5 kgCo and Mo each per 1 ha.

The vegetation period in the experimental area lasts from April to September (150–190 days). Meteorological conditions are variable, particularly concerning rainfall during the vegetation period. The range, after disregarding 25 % of excessive cases for annual precipitation, was from 733.2 to 990.0 mm for the years 1968–2008 and between 461.5–658.2 mm for the April–September period. Mean annual temperature was 5.86 ^{o}C.

Analysis of dehydrogenase activity was conducted in fresh soil material with natural water content. In order to determine the respiration activity collected soil material was dried in the open air and 3 weeks after collecting moistened to 30 % of maximum field moisture content. The biological activity was measured in the soil material by means of OxiTop Control measuring system [5].

Manometric measurement comprised change of pressure in a closed container (Fig. 2) which was proportional to oxygen consumption and created by respiratory processes taking place in the sample. The measurement time was 7 days and changes of respiration were registered automatically every 28 minutes (360 cycles). The equivalent amount of created CO_2 was absorbed by 1 mol · dm^{-3} NaOH solution.

Fig. 2. A photo of equipment for measuring a demand for oxygen (photo: M. Kopec)

The applied system consisted of 1 dm^3 sample bottles with equipment. For the period of measurement the sample bottles were placed in a thermostatic cabinet where the

constant temperature 20 °C (±0.1 °C) was maintained. The data from the measurement were sent to the controlling head via infra-red interface and then to the computer using Achat OC programme. Biological activity of the materials was expressed in $mgO_2 \cdot g^{-1}$ d.m. of soil and computed according to the formula:

$$BA = \frac{M_{O_2}}{R \cdot T} \cdot \frac{V_{fr}}{m_{Bt}} \cdot \left| \Delta p \right|$$

where: BA – biological activity,
M_{O_2} – oxygen molecular weight (31998 mg · mol^{-1}),
R – the gas constant (83.14 L · hPa) · (K · mol)$^{-1}$,
T – measurement temperature [K],
m_{Bt} – soil dry weight [kg],
$|\Delta p|$ – change of pressure [hPa],
V_{fr} – free gas volume calculated in the following way:

$$V_{fr} = V_{ges} - VAM - VBf$$

where: V_{ges} – total volume of sample bottle,
VAM – volume of the absorber and internal auxiliary equipment,
VBf – volume of moist soil.

Soil dehydrogenase activity was measured using Thalmann [6] methodology in soil with natural moisture content. Soil samples were incubated at the temperature of 37 °C for 24 hours with 1 % solution of triphenyltetrazolium chloride (TTC) as hydrogen ion acceptor. The applied method bases on spectrophotometric measurement of triphenyltetrazolium chloride (TTC) forming in result of reduction to triphenylphormazan (TPF) at 546 nm. The result of the measurement was converted according to prepared standard curve and expressed in TPF · kg^{-1} · d^{-1}.

Regression equations and standard deviations were computed on the basis of four object replications and one-way ANOVA was conducted using Excell or Statistica programmes.

Results and discussion

In case of respiratory activity computed on the basis of regression equation in time (x) we are dealing with two equation parameters:

$$y = ax + b$$

where: a – is the change occurring in a time unit,
b – the value of reaction in the first period of incubation or its post-effect.

Table 2

Equations of pressure changes in the 2nd and 4–7th days of measurements (x = 28 – minute cycle)

Fertilising objects	2nd day	R^2	7th day	R^2
PK	$y = -0.1137x - 5.655$	0.987	$y = -0.0676x - 16.93$	0.988
PK + Ca	$y = -0.1221x - 7.403$	0.992	$y = -0.0601x - 18.951$	0.988
90 kg N + PK	$y = -0.1090x - 6.021$	0.983	$y = -0.0584x - 16.948$	0.988
90 kg N + PK + Ca	$y = -0.1141x - 7.254$	0988	$y = -0.0574x - 18.131$	0.988
180 kg N + PK	$y = -0.0907x - 5.716$	0.977	$y = -0.0547x - 14.986$	0.987
180 kg N + PK + Ca	$y = -0.0899x - 6.821$	0.981	$y = -0.0545x - 15.822$	0.989

In respect of presented results, both parameters are important. On the basis of measurements presented in Fig. 3 it was established that about the 100th cycle, which corresponds to 47th hour (the end of the second 24 hour period) of incubation a noticeable change in the respiration rate occurred.

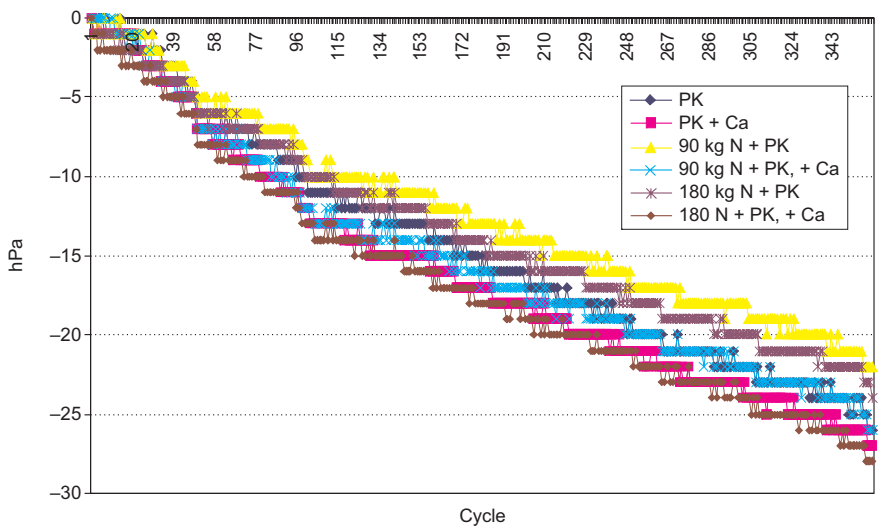

Fig. 3. Pressure changes during measurement conducted by Oxi Top Control

Table 3 presents average oxygen demand in the 2nd and 7th day of incubation and the indicator is expressed in $mgO_2 \cdot g^{-1}$ of soil d.m. and unit oxygen demand in these days converted into $mgO_2 \cdot g^{-1}$ d.m. $\cdot h^{-1}$.

In the 24 hour period unit oxygen demand was 1.66–2 times higher than on the 7th day. On the 7th day analysis of variance revealed greater diversification of homogenous groups than on the 2nd day, what means that the test required at least four-day incubation. Homogenous groups indicate a significant effect of fertilization on respiratory activity. Marked effect of liming on this indicator was observed. Intensive fertilization with 180 kg N + PK $\cdot$ ha^{-1} led to the conditions under which the soil

Table 3

Mean daily demand for oxygen [$mgO_2 \cdot g^{-1} \cdot d^{-1}$] and respiratory activity [$mgO_2 \cdot g^{-1} \cdot h^{-1}$] in the soil in the selected periods of incubation, dehydrogenaze activity [µg TPF $\cdot g^{-1} \cdot d^{-1}$] and soil pH

Fertilising object	Demand for oxygen [$mgO_2 \cdot g^{-1} \cdot d^{-1}$]		Respiratory activity [$mgO_2 \cdot g^{-1} \cdot h^{-1}$]		Dehydrogenaze activity		pH
	2nd day	7th day	2nd day	7th day	[µg TPF $\cdot g^{-1} \cdot d^{-1}$]	V [%]	
PK	2.759b	9.448d	0.079	0.047	18.56b	45.99	4.71b
PK + Ca	3.396d	9.679e	0.084	0.042	17.76ab	9.78	5.31b
90 kg N + PK	2.834bc	8.935c	0.076	0.041	18.03b	11.83	4.69ab
90 kg N + PK + Ca	3.28d	9.269d	0.079	0.039	20.62b	18.23	4.85b
180 kg N + PK	2.590a	8.353b	0.063	0.038	11.59a	20.67	4.12a
180 kg N + PK + Ca	2.942c	8.087a	0.063	0.038	14.49ab	24.93	4.84b

revealed the least oxygen demand and its unit consumption in time. In case of this object, relationships between the values in soil series without liming and limed were inverted.

A similar course of variability within the object is characteristic for all objects (Fig. 4).

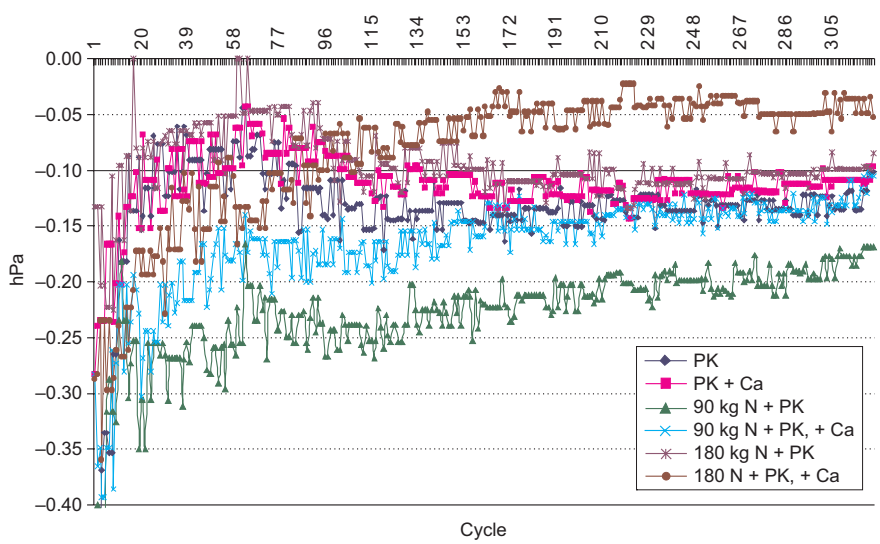

Fig. 4. Dynamics of changes of standard deviation (n = 4 repetitions) in comparison with arithmetic mean of pressure measurements for particular objects in the subsequent measurement cycles (x = 28 minutes)

In the initial phase of big changes of pressure about 50 cycles (*ie* about 1 day) the measurement became stabilized. In the final period of the analysis the variability was about 1/3 of the initial variability and was always lower within the object of the limed series. The share of deviation from 4 replications to the mean of 360 cycles in the last

cycle ranged from 5 % to 17 %. The lowest variability was noted between the replications of limed series from the 180 kg N + PK object and the highest in the soil from 90 kg N + PK object.

The highest dehydrogenase activity was registered in the object fertilized with 90 kg N + PK in the limed series. In the literature [4] fertilization is regarded as optimal for maintaining the land productivity over a long period of time. Dehydrogenase activity was on a similar level in soil of both series on the object fertilized with PK and in the soil of non-limed series 90 kg N + PK. Fertilization with 180 kg N + PK caused a decline in dehydrogenase activity by 30–35 % in comparison with the soil from the corresponding objects fertilized with 90 kg N + PK. In case of nitrogen treatment in the soil of limed series, dehydrogenase activity was higher by 14–25 % than assessed in the non-limed series. The average value of dehydrogenase activity in soil of the PK fertilized object in the non-limed series was slightly divergent from the observed tendency, which might have been connected with high variability among the replication of this object.

The analysed example does not allow to show an unanimous relationship between the soil pH (Table 3) and its biological activity. Under conditions of the analysed experiment fertilization is the factor more strongly determining the values of discussed soil properties. Jiang et al [7] demonstrated that biological activity is connected with soil aggregates. It may explain presented research results, because fertilization used in the experiment modified air and water soil properties from the individual experimental objects [8]. Presented research is important for the investigations on carbon sequestration. Wang et al [9] think that in a longer perspective, soil degradation decreases sequestration on grasslands. Previous papers demonstrated that fertilization with 180 kg N + PK led to monoculture of *Holcus mollis*, shallowing of the humus horizon and depletion of some microelements [4, 8, 10]. Sakowska et al [11] revealed that among agricultural crops, the legume stand revealed a markedly better productivity and carbon dioxide use in comparison with the unicotyledonous. These conditions may arise at balancing or increasing the proportion of white clover in result of nitrogen fertilization reduction and increasing soil abundance in phosphorus and potassium.

Conclusions

1. Long-term permanent mineral fertilization caused a significant change of soil biological activity.

2. Systematic soil liming caused that its biological activity was generally bigger than when this measure was not applied. However, liming diversified this feature to a lesser extent than mineral fertilization.

3. The highest values of respiration and dehydrogenase activity were registered in the soil of the object where balanced fertilization on the level of 90 kg N + PK was used and on the object where fertilization increased abundance in phosphorus and potassium leading to better biological nitrogen fixation.

4. The analysis based on the results of measurement on 4–7 day using manometric method is a sensitive indicator of soil respiratory activity.

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OCENA AKTYWNOŚCI BIOLOGICZNEJ GLEBY W WARUNKACH DŁUGOTRWAŁEGO ZRÓŻNICOWANEGO NAWOŻENIA MINERALNEGO

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Abstrakt: Współczesne modele sekwestracji węgla wymagają poznania aktywności biologicznej na różnych poziomach i przypadkach zakłócenia procesów oraz zależności przemian węgla i azotu w czasie. Jednym z elementów wpływających na aktywność respiracyjną są właściwości gleby ukształtowane nawożeniem. W warunkach długotrwałego doświadczenia nawozowego po 44 latach użytkowania górskiej runi łąkowej zbadano metodą manometryczną aktywność respiracyjną gleby. Do badań wyznaczono obiekt, w którym stosowano wyłącznie nawożenie fosforem i potasem oraz obiekty nawożone saletrą amonową w dwóch dawkach 90 i 180 kgN · ha^{-1} na tle PK. Doświadczenie wykonano z glebą serii wapnowanej i bez wapnowania. Pomiar manometryczny obejmował zmianę ciśnienia w zamkniętym naczyniu, która jest proporcjonalna do zużycia tlenu przez próbkę a powstaje w wyniku zachodzących w niej procesów oddychania. Powstające równoważne ilości CO_2 były absorbowane przez roztwór NaOH o stężeniu 1 mol · dm^{-3}. Aktywność biologiczną materiałów wyrażono w $mgO_2 \cdot g^{-1}$ s.m. · d^{-1}. Równania zmian ciśnienia (y) wskazują na różnice respiracji gleby spowodowane intensyfikacją nawożenia. Systematyczne wapnowanie gleby spowodowało, że jej aktywność biologiczna była na ogół większa niż w przypadku braku tego zabiegu. Wapnowanie jednak w mniejszym stopniu niż nawożenie mineralne różnicowało tę cechę. Największe

wartości aktywności respiracyjnej oraz aktywności dehydrogenaz (μg TPF · g^1 ś.m. · d^{-1}) stwierdzono w glebie obiektu, w którym stosowano zrównoważone nawożenie na poziomie 90 kg N + PK oraz obiektu, w którym zwiększono w wyniku nawożenia zasobność w fosfor i potas, powodując zwiększenie biologicznego wiązania azotu.

Słowa kluczowe: aktywność biologiczna, gleba, długotrwałe doświadczenie