

# EFFECT OF CROP ROTATION AND LONG TERM FERTILIZATION ON THE CARBON AND GLOMALIN CONTENT IN THE SOIL

## WPŁYW ZMIANOWANIA I WIELOLETNIEGO NAWOŻENIA NA ZAWARTOŚĆ WĘGLA I GLOMALINY W GLEBIE

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### ABSTRACT

The research was performed on the basis of soil samples taken from a multi-year long fertilization experiment carried out in Skieriewice. The source of samples was soil under potato and rye cultivated in monoculture and in the 5-fields rotation system. The following combinations of fertilization were concerned: Ca, NPK and CaNPK (doses since 1976: 1.6 t·ha<sup>-1</sup> CaO every 4 years in monoculture and 2 t·ha<sup>-1</sup> CaO every 5 years in crop rotation, 90 kg·ha<sup>-1</sup> N, 26 kg·ha<sup>-1</sup> P, 91 kg·ha<sup>-1</sup> K). Laboratory analyzes involved determination of total organic carbon (TOC) and glomalin operationally described as a total glomalin related soil protein (TGRSP). It was found that regardless of cultivated plants and the method of fertilization, only cultivation system such as rotation and monoculture significantly influenced the content of TGRSP. TOC was significantly influenced by interaction between species of cultivated plant and the system of cultivation. The analyzed factors within the method of cultivation (monoculture and crop rotation) did not influence significantly the TGRSP content while cultivated plant species, in monoculture, significantly influenced on TOC content. There was also noted positive correlation ( $r = 0.72$ ) between TGRSP and TOC.

**Keywords:** crop rotation, glomalin, monoculture, soil organic carbon

### STRESZCZENIE

Badania prowadzono w oparciu o próbki gleby z poletek Wieloletniego Statycznego Doświadczenia Nawozowego w Skieriewicach spod uprawy ziemniaka i żyta w monokulturze i zmianowaniu 5-polowym. Uwzględniono następujące wybrane kombinacje nawozowe: Ca, NPK i CaNPK (dawki od 1976 roku: co 4 lata 1,6 t·ha<sup>-1</sup> CaO w monokultuze i co 5 lat pod jęczmien w ilości 2 t·ha<sup>-1</sup> CaO w zmianowaniu oraz 90 kg·ha<sup>-1</sup> N, 26 kg·ha<sup>-1</sup> P, 91 kg·ha<sup>-1</sup> K). W glebach oznaczano zawartość ogólnego

węgla organicznego (TOC) oraz glomaliny określonej roboczo jako proteiny pochodne glomaliny ogółem (TGRSP). Stwierdzono, że jedynie system uprawy tj. zmianowanie lub monokultura, niezależnie od uprawianej rośliny oraz sposobu nawożenia, istotnie wpływały na zawartość TGRSP. Natomiast na zawartość TOC w glebie istotnie wpływała interakcja pomiędzy systemem uprawy, a gatunkiem uprawianej rośliny. Analizowane czynniki w obrębie systemu uprawy nie wpływały istotnie na zawartość TGRSP, natomiast w zakresie TOC, w monokulturze, zanotowano istotny wpływ gatunku uprawianej rośliny. Zaobserwowano także dodatnią korelację ( $r = 0,72$ ) pomiędzy zawartością TGRSP oraz TOC.

**Słowa kluczowe:** zmianowanie, glomalina, monokultura, materia organiczna gleby

## DETAILED ABSTRACT

W niniejszej pracy badano wpływ rodzaju nawożenia mineralno-organicznego na zawartość węgla organicznego (TOC) i ogółem protein glebowych pochodnych glomaliny (TGRSP) w glebie spod uprawy ziemniaka i żyta w monokulturze i zmianowaniu. Próby do analiz pobrano z obiektów wieloletniego, statycznego doświadczenia nawozowego SGGW prowadzonego w Skiernewicach. Gleba pola doświadczalnego należy do typu płowego, opadowo-glejowego wg WRB (2006) "Stagnic Luvisols". Próby glebowe pobrano z warstwy 0-25 cm w 75 roku trwania doświadczenia. Z doświadczenia wybrano obiekty nawożone Ca, NPK i CaNPK, na których uprawiano żyto i ziemniaki w monokulturze i w zmianowaniu 5-polowym. Próbki gleby z poletek uwzględniających zmianowanie (ziemniak 30 t·ha<sup>-1</sup> obornika, jęczmień jary, konicyzna czerwona, pszenica, żyto) pobrano po zbiorze ziemniaka z poletek nawożonych obornikiem jesienią poprzedniego roku oraz po zbiorze żyta, w piątym roku po oborniku. Nawozy mineralne były stosowane w jednakowych dawkach na wszystkich polach wybranych kombinacji w pięciu powtórzeniach: 90 kg·ha<sup>-1</sup> N, 26 kg·ha<sup>-1</sup> P i 91 kg·ha<sup>-1</sup> K. Stosowanie co 4 lata 1,6 t·ha<sup>-1</sup> CaO (monokultura) oraz co 5 lat pod jęczmień w ilości 2 t·ha<sup>-1</sup> CaO (zmianowanie), ma na celu utrzymanie stanu zakwaszenia gleby na optymalnym poziomie (pH 4,25- 6,36).

W próbach zbiorczych materiału glebowego (średniej z 5-powtórzeń), oznaczono zawartość węgla organicznego (Corg) w g·kg<sup>-1</sup> gleby analizatorem CHN Model 1106 firmy Carlo-Erba Strumentazione. Ekstrakcję glomaliny z gleb wykonano według metodyki opracowanej przez Sarę Wright (Wright and Upadhyaya, 1996; Wright et al., 2006). Ekstrakcja gleby polega na autoklawowaniu odważonej próbki w roztworze cytrynianu sodu. W skrócie proces ekstrakcji polegał na odważeniu 1 g gleby do 50 ml polipropylenowej probówki przeznaczonej do wirowania, dodaniu 50 mM cytrynianu sodu (pH 8,0), autoklawowaniu ( $t = 121^\circ\text{C}$ ,  $p = 1,4 \text{ kgcm}^{-1}$ ), odwirowaniu i zlaniu płynu nadosadowego. Wszystkie kroki ekstrakcji powtarzano aż do uzyskania prawie bezbarwnego supernatantu.

W związku z porównaniem wyników z jednego powtórzenia doświadczenia analizę statystyczną wyników w obrębie monokultury i zmianowania przeprowadzono dla układu doświadczenia dwuczynnikowego pojedynczego, bezpowtórzeniowego w układzie całkowicie losowym. Czynnik pierwszy - gatunek uprawianej rośliny występował na dwóch poziomach (ziemniak, żyto), czynnik drugi – rodzaj nawożenia występował na 3 poziomach (Ca, NPK i CaNPK). Wyniki porównano także stosując układ doświadczenia 3 czynnikowego, gdzie trzecim czynnikiem, występującym na 2 poziomach był system uprawy tj. monokultura i zmianowanie 5 polowe.

W badaniach stwierdzono, iż wyższa średnia zawartość TGRSP występowała w glebie pod żywem, a na jej zawartość istotnie wpływał system uprawy. Gleba spod upraw w zmianowaniu charakteryzowała się istotnie wyższą zawartością TGRSP w stosunku do gleby spod upraw w monokulturze. Natomiast różnice w zawartości TGRSP spowodowane sposobem nawożenia, a także gatunkiem uprawianej rośliny były nieistotne. W wyniku przeprowadzonej analizy statystycznej nie stwierdzono istotnego wpływu czynników doświadczenia na zawartość całkowitego węgla organicznego (TOC) w badanej glebie spod uprawy żyta i ziemniaka. Stwierdzono natomiast istotne interakcje pomiędzy gatunkiem uprawianej rośliny, a systemem uprawy na zawartość TOC. W celu określenia związku pomiędzy zawartością TOC i TGRSP obliczono współczynnik korelacji Pearsona ( $r = 0,72$ ), który wskazuje na silną pozytywną korelację zachodzącą pomiędzy analizowanymi wartościami.

Na podstawie wykonanych analiz stwierdzono, że większa zawartość TGRSP w glebie może być dobrym wskaźnikiem zawartości trudniej utlenialnych frakcji węgla nie związanej bezpośrednio z materią organiczną wprowadzaną na przykład z obornikiem lub resztkami pozbiorowymi. W odróżnieniu od analizy TOC, który może opisywać zasobność pola w materię organiczną w odniesieniu do krótkiego okresu czasu – np. szybki wzrost po zastosowaniu obornika, zawartość TGRSP będzie zwiększać się powoli wraz z postępującą kolonizacją korzeni i przestrzeni między glebowych przez strzępki grzybów AM. Wykonane analizy potwierdzają istnienie pozytywnej korelacji pomiędzy zawartością TOC i TGRSP, co może świadczyć o korzystnym wpływie wprowadzania do gleby „świeżej” materii organicznej. Wykazano również, że zmianowanie w porównaniu z monokulturą wpływa istotnie korzystniej na zawartość TGRSP w glebie.

## INTRODUCTION

The potential fertility of the soil is determined on the base of the general content of soil organic matter (SOM). In the agricultural practice, it is important to determine the contribution of fractions responsible for the progressive transformation associated with the different susceptibility to degradation. Apart from the most labile fraction, which is a source of nutrients, fractions resistant to degradation that decide on the preserving and reconstructing of humus resources are important. The example of such substance is glomalin, which was shown to account for 25 and 52% of the total C in the mineral soils and organic soils, respectively (Schindler, at al., 2007).

Glomalin is a biochemically defined glycoprotein produced by arbuscular mycorrhizal fungi (AMF), measured operationally in soils as glomalin-related soil protein (GRSP) (Driver, at al., 2005). Glomalin has been found in abundance from 2 to 15 mg g<sup>-1</sup> and up to >60 mg g<sup>-1</sup> in a wide range of soil types (Nichols, 2003). It was reported that glomalin is present in soils in concentrations that are up to four times as great as humic acid concentrations (Schindler, at al., 2007). Ubiquity of glomalin, coupled with its apparent recalcitrance and its consequent long residence time in soil, can result in glomalin comprising large pools of soil carbon and nitrogen (Janos, at al., 2008). Until glomalin discovery in 1996 by soil scientist Sara F. Wright, it was mistaken for an unidentifiable constituent of soil organic matter (Comis, 2002). Depending on the analyzed components of humus fractionation the fractioning is based on the appropriately selected solvents, in case of glomalin it is sodium citrate (Wright, at al., 2006; Rosier, at al., 2006).

In the present study, there was investigated the influence of the type of mineral-organic fertilization on the content of organic carbon (TOC) and total glomalin-related

soil protein (TGRSP) in soil under potatoes and rye cultivated in monoculture and rotation.

Increased content of TGRSP in soil could be a good indicator of the content of not easily susceptible to oxidation carbon fraction, which is not straight related with organic matter applied into the soil with manure or crop residues. In contrast to the analysis of TOC, which can describe the abundance of organic substance in the field for a short period of time - for example, rapid growth after the application of manure, TGRSP content will increase gradually with the progressive colonization of the roots and the space between the soil by the hyphae of AM fungi.

## MATERIAL AND METHODS

The soil samples for analyzes were taken from multi-year long, static, fertilization experiment carried out by SGGW in Skierniewice Research Station. According to WRB (2006) the soil from experimental plots belongs to stagnic luvisols. The soil samples were taken from the depth of 0-25 cm in the 75<sup>th</sup> year of the experiment, from the selected plots fertilized with Ca, NPK and CaNPK where rye and potatoes were grown in monoculture and in the 5-field rotation system. Soil samples from the plots in the 5-fields rotation system (potato 30 t·ha<sup>-1</sup> manure, spring barley, red clover, winter wheat, winter rye) were taken after potato crop (from plots fertilized with manure, autumn previous year) and after rye crop. The complete scheme of fertilization and crop rotation of long lasting experiments in Skierniewice is presented in Mercik's publication (Mercik, at al. 1999). Mineral fertilizers were applied in equal doses in all plots of selected combinations of the five replications. Application of 1,6 t·ha<sup>-1</sup> CaO every four years (monoculture) and 2 t·ha<sup>-1</sup> every five years under barley (crop rotation) was intended to balance the soil pH to the optimal level of 4.25-6.36 (Mercik, 2000). The annual doses of NPK were 90 kg·ha<sup>-1</sup> N, 26 kg·ha<sup>-1</sup> P and 91 kg·ha<sup>-1</sup> K. The bulk soil samples (average of 5 replications) were analyzed for organic carbon content (TOC) [g·kg<sup>-1</sup>] by CHN analyzer, model 1106 Carlo-Erba Strumentazione.

The extraction of glomalin (TGRSP) from soil samples was performed according to protocol established by Sara Wright (Wright and Upadhyaya, 1996; Wright at al., 2006). Soil extraction include autoclaving of weighed sample in a solution of sodium citrate. Briefly, the extraction process consists of following steps: weighing 1 g of soil and placing it in the 50 ml PP centrifuge tube, adding 50 mM sodium citrate (pH 8.0), autoclaving ( $t = 121^\circ\text{C}$ ,  $p = 1.4 \text{ kg}\cdot\text{cm}^{-2}$ ), centrifuging and decanting of the supernatant. All above-mentioned steps were repeated until the supernatant was almost colorless.

The TGRP content was analyzed with utilization of Bio-Rad protein dye reagent (Bio-Rad 500-0006), which basic component is Coomassie brilliant blue used in the original Bradford protocol (Bradford, 1976). Extract's absorbance was measured at 595 nm by UV-VIS SmartSpec spectrophotometer (Bio-Rad 170-2525). Disposable 50 µl cuvettes were used. Calibration curve was prepared based on ready solutions (Bio-Rad 500-0207) of bovine serum albumin (BSA) according to producer's protocol. When necessary, extracts were diluted by phosphate buffer saline (PBS) pH 7.4.

Because the obtained analysis results originated from one repetition of experiment, the statistical analysis of results within the monoculture and rotation were performed for the single, two factor, non-replicated, completely random configuration of experiment. The first experimental factor was cultivated plant species (potato, rye),

the second factor was fertilization (Ca, NPK and CaNPK). The results were also compared within the three-factor system, where third factor was a cultivation system (monoculture and five fields rotation). Analysis of variance was performed using the software ANALWAR by prof. F. Rudnicki.

## RESULTS AND DISCUSSION

It was found that higher average content of TGRSP occurred in the soil under rye. The content of TGRSP was significantly affected by the cultivation system: monoculture and crop rotation. Soil from crop rotation was characterized by a significantly higher proportion of TGRSP in relation to the soil from the crops in monoculture. However, differences in TGRSP content caused by fertilization and plant species were insignificant. There was also no significant interaction between the analyzed factors (Table 1). Analyze of TGRSP content only within the particular cultivation system (two factor variance analysis) indicate no significant differences caused by fertilization method or cultivated plants.

Table 1: Total glomalin related soil proteins (TGRSP) content in the soil [g·kg<sup>-1</sup>] under potato and rye cultivated in monoculture and rotation

Tabela 1: Zawartość ogółem protein pochodnych glomaliny (TGRSP) [g·kg<sup>-1</sup>] w glebie spod ziemniaka i żyta uprawianych w monokulturze i zmianowaniu

Factors Czynniki		B Plant B Roślina		Mean Średnia
A Cultivation system System uprawy	C Fertilization Nawożenie	Potato Ziemniak	Rye Żyto	
Monoculture Monokultura	Ca	1.739	1.893	1.816
	NPK	1.007	2.611	1.809
	Ca+NPK	1.486	1.610	1.548
	mean	1.411	2.038	1.724
Crop rotation Zmianowanie	Ca	2.594	2.426	2.510
	NPK	2.168	2.963	2.566
	Ca+NPK	2.556	2.885	2.721
	mean	2.439	2.758	2.599
Mean Średnia	Ca	2.167	2.160	2.163
	NPK	1.588	2.787	2.187
	Ca+NPK	2.021	2.248	2.134
	mean	1.925	2.398	2.162

LSD<sub>a=0,05</sub> (Tukey test):

A= 0.384

B= n.s.

C= n.s.

A/B= n.s.

B/A= n.s.

A/C= n.s.

n.s. – not significant

C/A= n.s.      B/C= n.s.      C/B= n.s.

The differences within separately analyzed cultivation systems were not significant.

The lower TGRSP content in the soil under potato probably results from the need of more drastic agrotechnical treatments aimed at the formation of field ridges. The researches concerning corn cultivation (Galvez, et al., 2001) indicate that farming systems with reduced tillage is favorable to greater root colonization by AM fungi. Higher content of TGRSP in the crop rotation system corresponds to observations (Gosling, et al., 2006) that increase of crop bio diversity is beneficial for the presence of AM fungi, which in turn is related with production and appearance of glomalin (Rosier, et al., 2006; Driver, et al., 2005).

The statistic analysis showed that experimental factors did not influence significantly TOC content in the soil under potato and rye. However, the significant interaction

between plant species and cultivation system, that influenced TOC content, was revealed (Table 2).

Table 2 Total organic carbon (TOC) content [ $\text{g}\cdot\text{kg}^{-1}$ ] in the soil under potato and rye cultivated in monoculture and rotation

Tabela 2 Zawartość węgla organicznego ogółem (TOC) [ $\text{g}\cdot\text{kg}^{-1}$ ] w glebie spod ziemniaka i żyta uprawianych w monokulturze i zmianowaniu

Factors Czynniki		B Plant B Roślina		Mean Średnia
A Cultivation system System uprawy	C Fertilization Nawożenie	Potato Ziemniak	Rye Żyto	
Monoculture Monokultura	Ca	7.6	9.7	8.65
	NPK	5.4	8.8	7.10
	Ca+NPK	5.5	9.9	7.70
	mean	6.17	9.47	7.82
Crop rotation Zmianowanie	Ca	9.7	8.6	9.15
	NPK	9.9	9.7	9.80
	Ca+NPK	10.1	9.9	10.00
	mean	9.90	9.40	9.65
Mean Średnia	Ca	8.65	9.15	8.90
	NPK	7.65	9.25	8.45
	Ca+NPK	7.80	9.90	8.85
	mean	8.033	9.433	8.033
LSD <sub>a=0,05</sub> (Tukey test):		A= n.i. A/B= 1.267 C/A= n.i.	B= n.i. B/A= 1.267 B/C= n.i.	C= n.i. A/C= n.i. C/B= n.i.

Variance analysis in monoculture

LSD<sub>a=0,05</sub> (Tukey test): B= 2.538 C= n.i.

Variance analysis in crop rotation

LSD<sub>a=0,05</sub> (Tukey test): B= n.i. C= n.i.

\* doses since 1976: 1.6 t·ha<sup>-1</sup> CaO every 4 years (monoculture) and 2 t·ha<sup>-1</sup> CaO every 5 years (crop rotation), 90 kg·ha<sup>-1</sup> N, 26 kg·ha<sup>-1</sup> P, 91 kg·ha<sup>-1</sup> K

The mean TOC content in the soil under rye in monoculture ( $9.47\text{ g}\cdot\text{kg}^{-1}$ ) was about 54% higher than in the soil under potato ( $6.17\text{ g}\cdot\text{kg}^{-1}$ ). Among the rye plots the lowest TOC content was noted in the soil from objects fertilized with NPK ( $8.8 \text{ g}\cdot\text{kg}^{-1}$ ), however, it was higher in the objects fertilized with Ca and CaNPK ( $9.7$  and  $9.9 \text{ g}\cdot\text{kg}^{-1}$ ), but the differences were not significant. Mineral fertilization also caused a reduction of TOC in the soil under potato. Every 4 years Ca application stabilized TOC content in the soil under the rye. The higher TOC content was observed in the limed object than in plots in which Ca was not applied. This phenomena is explained (Mercik and Stępień, 1996) by inhibition of organic substances leaching and by slower mineralization of SOM at higher pH. When analyzing 5 fields crop rotation system, there were no significant differences in the TOC content in the soil under potato nor rye (Table 2). The mean TOC content in the soil under potato was  $9.9 \text{ g}\cdot\text{kg}^{-1}$  and was higher by about 5.3% than in the soil under rye ( $9.4 \text{ g}\cdot\text{kg}^{-1}$ ). Obtained results of lower TOC content in the soil under rye, in crop rotation, might be the consequences of crop sequence (Cieścińska and Dębska, 2009). Rye was cultivated in the last, fifth year, after manure application (effect of natural fertilizer was already weakened) and in the second year after fabaceae plants. The soil samples from potato plots were taken from objects that had been fertilized with manure in the previous autumn. When considering cultivation system, higher mean TOC

concentration was noted in the soil under rye in monoculture ( $9.47 \text{ g}\cdot\text{kg}^{-1}$ ) and in the crop rotation system, inversely, in the soil under potato ( $9.90 \text{ g}\cdot\text{kg}^{-1}$ ). Comparing mean TOC content in the soil from plots fertilized with Ca, NPK and CaNPK in the monoculture and rotation system ( $7.82$  and  $9.65 \text{ g}\cdot\text{kg}^{-1}$ ), about 24% higher mean content was noted in the rotation system. The rotation alongside the intensity of organic fertilization (manure), contributes positively to the quality and quantity of crop residues that remain on the fields (Gonet, 1997; Zaujec 1980). It is therefore assumed that the rate of mineralization or humification of manure depend mainly on agricultural practices and crops grown.

In order to determine the relationship between the content of TOC and TGRSP Pearson correlation coefficient ( $r = 0.72$ ) was calculated. It indicates a strong positive correlation between the analyzed values, which coincide with observations made previously (Wilson, et al., 2009) and also confirms that glomalin may be an important pool of soil carbon.

## CONCLUSIONS

It was found that the cultivation system (monoculture and rotation) significantly affects soil TGRSP content and interact with cultivated plants (potato, rye) on the TOC content. Thus, the use of crop rotation is beneficial for both the content of TOC as well as TGRSP in soil.

The presented study also reveals that the analyzed methods of fertilization and species of crops were not significant factors influencing TGRSP and TOC content in the soil, except that in monoculture TOC content was significantly affected by plant species.

Performed analysis confirms the existence of a positive correlation between TOC content and TGRSP, which may indicate a beneficial effect of the introduction into the soil "fresh" organic matter. It was also demonstrated that the rotation compared to monoculture significantly, positively affect the content TGRSP in the soil.

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