Chapter 9

Effect of Crop Rotation and Nitrogen Fertilization on the Quality and Quantity of Soil Organic Matter

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Additional information is available at the end of the chapter

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1. Introduction

Soil organic matter (SOM) is one of the most important features of the soil. Its characteristic depends on a variety of biotic and abiotic variables of the ecosystem, such as climate, soil texture, mineral composition, quantity of organic residues and other factors. Currently, in an era of rapidly changing civilization, leading to changes in climate and soil conditions, SOM content becomes increasingly important, not only for the proper functioning of ecosystems, but also for socio-economic development of many regions of the world [1, 2]. In the first half of the past century, there were hardly contradictions between the agricultural cultivation and the environment. The substance circulations were closed, animal production comparatively small and mainly regularly allocated. The mineral fertilization was only used to a slight extent. A fundamental change has taken place during the last decades. With the increasing use of mineral fertilizers, yields have increased by more than 100% thus the quantity of roots and harvest residues on the field has increased strongly as a source of organic matter. However, in agricultural practice they are commonly removed from the field after harvest resulting in SOMdecrease.

During the last ten years in EU countries, the progressive degradation of SOM is observed. Thus, this issue was reflected in the EU soil strategy (COM (2002) 179), on which the actual reduction of soil organic matter content was listed as one of the most important problems. In Poland, the reduction of SOM content in soils became a problem particularly significant.

Nearly the whole territory of Poland (99,7%), covering about 313 thousands square kilometres is situated in the Baltic Sea basin. This territory is drainaged by two big rivers Vistula and Odra and seven small rivers flowing directly to the sea. Natural farming condition in Poland are poor, due to prevalence of light, sand-derived soils (60% very light and light soils) and unfavourable climate. Due to soil texture and acid or very acid reaction more than

60% of the soils in Poland can be classified as soils witha relatively low content of organic matter. According to the newest survey 7,6% of arable soils show low content of SOM (below 1%), 47,1% of soils presentmedium content (1,1-2,0%), 29,3%, high content (2,1-3%) and only 3% of soils show very high content of organic matter (above 3%) [3]. The percentage of acid and very acid soils is very high and exceeds 50%, and soil acidity seems to be one of the most important factors leading to degradation of the quantity and quality of organic matter [4]. The summaryof the last four-years period of agrochemical soils monitoring program reveals that 20,2% of soils are vey acid (pH_{KC}l below 4,5) and 29,4% acid (pH_{KCl} 4,5-5,5) [4].

Besides, the intensification of soil use combined with the simplified crop rotation and predominance of cereals together with an expansion of farming systems based on crop production with a reduced number of livestock or without animals intensifies the process of organic matter degradation [5- 7].

The turn over of organic substance are time dependent and become most apparent after decades. Therefore, long-term field experiments are a necessary tool for tracking changes in organic carbon content in the soil. In Europe, the most famous long-term experiences with testing of different mineral and natural fertilisers and/or cultivation of various plant species were held at Rothamsted (England), Halle and Bad Laustädt (Germany), Prague-Ruzyne (Czech Republic) and Skierniewice (Poland) [8].

The Institute of Soil Scienceand Plant Cultivation National Research Institute (IUNG-PIB) in Puławy is also involved in several kinds of long term field studies, including SOM content monitoring, since 1979. A special trait of these experiments is that crop rotations included plants enriching and exhausting soil from humus, have been apermanent factor for the last 33 years.

The objectives of presented paper was to access the impact of mineral nitrogen fertilization, manure application and crop rotation on the quantity and quantity soil organic matter in long term field experiment.

2. Composition of soil organic matter and methods of humus substances fractionation

Humic substances (HS) is the major organic constituents of soil. Humic substances are a mixture of particles that differ in their structure, mass, size, elemental composition and properties. The first record of humic acids extration by Achard dates back to 1786, and the first major study was done by Sprengel in 1820 [in 9]. Despite such a long research history on humic substances, there has been constant dispute over the structure of humus. There is an increasing interest in HS, because of their capacity for complexing metal ions, involvement in the organic geochemical cycle, affecting bioavailability and ecological effects of nutrient in water and stabilizing soil fertility.

The content and quality of humus are directly and indirectly determined by physical, chemical, biological and environmental properties. SOM quality is determined mainly by the percentage share of humic substances in soil. Humic substances can be subdivided into three major fractions: humic acids, fulvic acids and humins. Electron microscope observations reveal the humic acids of different soils to have a polymeric structure, appearing in form of rings, chains, and clusters. The size of their macromolecules ranges from 60 to 500 A and is mainly decided by the occurring humification process, which also exerts an influence on their spatial structure. Some of the main features of humic substances are shown in Figure 1 [10].

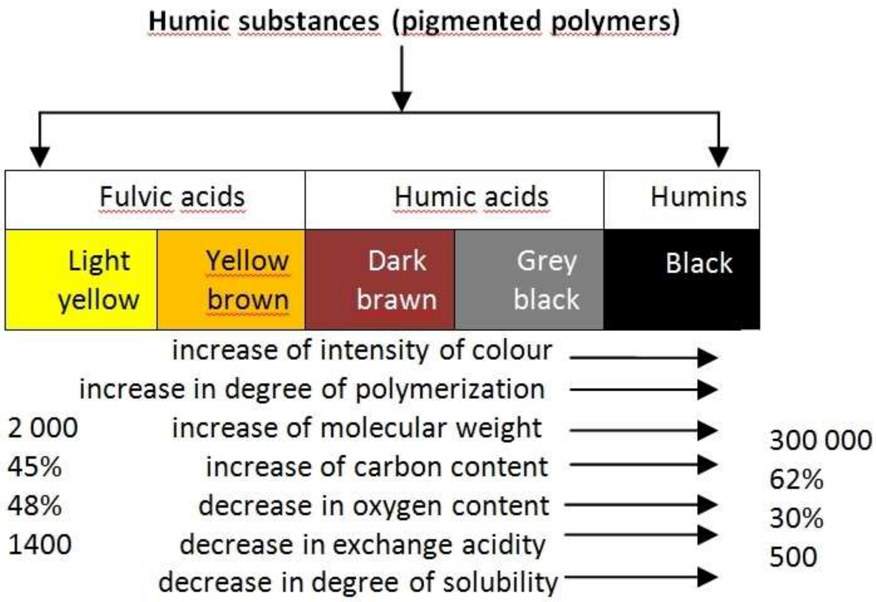

Figure 1. Chemical properties of humic substances.

Humic acids (HA) comprise a mixture of weak aliphatic and aromatic organic acids, containing carboxyl and phenolate groups. HA are not soluble in water under acidic conditions but soluble in water under alkaline conditions and precipitated from aqueous solutions when the pH decreases below 2. HA tend to be more aromatic and more prone to precipitation under the acid conditions common in soils, making them less mobile.

Fulvic acids (FA) are a humic substances soluble in water at any pH. They areless aromatic than humic acids. This part of humus remains in solution after removal of humic acids by acidification. FA have an oxygen content twice that of humic acids thus revealing a more acidic character than that of HA. The exchange capacity of FA is more than double that of HA.

Humins are the fraction of humic substances, not extracted from soil with either a strong base or a strong acid. Humins present within the soil are the most resistant to decomposition (slow to break down) of all the humic substance.This fraction plays a key role in soil fertility by improving structure and soil water capacity or providing a reservoir for the plant nutrients.

There are different ways to separate humic substances into each particular fraction. Every technique has its own advantages and limitations. The reagents used for extraction of humic acids from the soil are listed in table 1.

The most popular method is to use NaOH to extract humic acids from the soil. It is evident that extraction from soil with NaOH solution leads to the recovery of approximately two-thirds of the soil organic matter [6]. The amount of organic matter extracted from soil with the caustic alkali increase with time of extraction. Humic acids extracted by alkali solutions are characterized by high purity what is what is necessary for further physicochemical analyzes (UV-VIS, NMR and IR) [11].

Another reagent used for extraction of soil humusis sodium pyrophosphate ($Na_4P_2O_7$). Its advantage is that the auto oxidation process that occurs during the extraction of humic material is less intensive than with NaOH. However, this reagent allows to isolate only humus compounds loosely linked with the mineral fraction of the soil.

Type of material	Extractant	Organic matter extracted
Humic substances	NaOH Mild extractants	to 80%
	$Na_4P_2O_7$ and other Organic chelates:acetyloacetone, cupferron, hydroxyquinonline Formic acid (HCOOH)	to 30% to 30% to 55%

Table 1. Reagents commonly used for extration of organic constituents from soil [6]

The most common methods of humus fractionation are Schnitzers method and he standard, prior to extraction from the soil is determined by total organic carbon, by one of the recommended methods.

Schnitzer and Turin methods based on NaOH reagent are suitable for soils without carbonate thus the carbonate determination in soil is needed before [12-13]. In both methods, diluted sodium hydroxide is used after decalcification in mineral acid solution, which burnts the conjunctions of humic acids with calcium. Afterwards, humic and fulvic acids pass to the solution during alkaline extraction. On the base of the difference between the quantity of humus fractions before and without decalcification, the calculation of humic and fulvic acids associated with calcium is possible.

The separated fractions of humic substances have been recognized as the main indicators of soil fertility. The quality of SOM can be evaluated by determining the ratio of humic acids to fulvic acids (HA:FA) or carbon of humic acids to carbon of fulvic acids (CHA:CFA). It is widely described that fertile soils are characterized by higher humus content and CHA:CFA ratio>1.On the agricultural lands, soil humus properties are mostly determined by post- harvest residue left after the harvest of crops [8, 14]. Legumes increase organic matter content in soil. This is a consequence of chemical composition of organic material. Cereal straw contains more lignin, and legumes one more cellulose and nitrogen. Thus, the mineralization process of legumes residues occurs faster and the C:N ratio is narrower as compared to other crops [15].

The percentage of humus which occurs in the various humic fractions varies considerably from one soil type to another (Table 2) [16].

Soil	Humic acid/ Fulvic acid ratio	Soil	Humic acid/ Fulvic acid ratio
Chernozemordinary	2.0 – 2.5	Gray forest	1.0
Chernozem deep	1.7	Sod podzolic	0.8
Chestnut dark	1.5 – 1.7	Tundra	0.3

Table 2. Humic acid/fulvic acid ratios of some surface soils

A further attempt is made to investigate the properties of humic acids focusing on their spectroscopic characterization by UV–VIS and fluorescence spectra. This method is an important tool for determining the differences in humic substances structure, maturity and condensation degree [16, 17]. Measuring of optical properties of humic acids (absorbance and absorbance ratio) of alkaline soil extract allows to determine the degree of humification process and humic substances quality. Humic substances from various types of soil differ in the absorbance ratio. The measurement of absorbance is made in the wavelength range 280-665. The absorbance at wavelength 280 nm indicates the high content of lignin, 465 nm - young humic substances components associated with the first phase of humification process. Absorbance at 665 nm is related to well humified components [18].

The intensity of absorbance at wave length 280, 465 and 665 nm is used to calculate the ratiosA 2/4 (the ratio of absorbance in the wave length 280 nm to 465 nm), A 2/6 (280 nm to 665 nm) and A4/6 (465 nm to 665 nm). The absorbance ratio allows to recognize the structure of humus components. Larger values of 465 nm to 665 nm (A4/A6 ratio) are associated with the presence of smaller size organic molecules or more aliphatic structures and usually with higher content of functional groups [19].

2. Materials and methods

The study was conducted on the basis of a three factorial long-term field experiment carried on since 1979 at the Experimental Station Grabów of the Institute of Soil Science and Plant Cultivation in Puławy, on typical soil in Poland, classified as light loamy and sand texture according to USDA soil classification. The experiment was conducted with two crop rotations (I factor): A – recognized assoil exhausting from humus (potatoes, winter wheat, spring barley and corn for silage) and B –considered to enrich soil with humus (potatoes, winter wheat and mustard* as aftercrop for ploughing, spring barley with undersown** and clover with grass mixture). The experiment was performed in the split – block layout in two cycles moved by one year (Table 3).

Rotation	Year	Crop rotation A		Crop rotation B	
		Cycle 1	Cycle 2	Cycle 1	Cycle 2
I	1980	potatoes		potatoes	
	1981	winter wheat	potatoes	winter wheat*	potatoes
	1982	spring barley	winter wheat	spring barley**	winter wheat
	1983	maize for silage	spring barley	clover-grasses mixture	spring barley
	1984		maize for silage		clover-grasses mixture
II	1984	potatoes		potatoes	
	1985	winter wheat	potatoes	winter wheat*	potatoes
	1986	spring barley	winter wheat	spring barley**	winter wheat
	1987	maize for silage	spring barley	clover-grasses mixture	spring barley
	1988		maize for silage		clover-grasses mixture
III	1988	potatoes		potatoes	
	1989	winter wheat	potatoes	winter wheat*	potatoes
	1990	spring barley	winter wheat	spring barley**	winter wheat
	1991	maize for silage	spring barley	clover-grasses mixture	spring barley
	1992		maize for silage		clover-grasses mixture
IV	1992	potatoes		potatoes	
	1993	winter wheat	potatoes	winter wheat*	potatoes
	1994	spring barley	winter wheat	spring barley**	winter wheat
	1995	maize for silage	spring barley	clover-grasses mixture	spring barley
	1996		maize for silage		clover-grasses mixture
V	1996	potatoes		potatoes	
	1997	winter wheat	potatoes	winter wheat*	potatoes
	1998	spring barley	winter wheat	spring barley**	winter wheat
	1999	maize for silage	spring barley	clover-grasses mixture	spring barley
	2000		maize for silage		clover-grasses mixture
VI	2000	potatoes		potatoes	
	2001	winter wheat	potatoes	winter wheat*	potatoes
	2002	spring barley	winter wheat	spring barley**	winter wheat
	2003	maize for silage	spring barley	clover-grasses mixture	spring barley

	2004		maize for silage		clover-grasses mixture
VII	2004	potatoes		potatoes	
	2005	winter wheat	potatoes	winter wheat*	potatoes
	2006	spring barley	winter wheat	spring barley**	winter wheat
	2007	maize for silage	spring barley	clover-grasses mixture	spring barley
	2008		maize for silage		clover-grasses mixture

Table 3. The scheme of the experiment; ▭ Soil sampling term for SOM quality evaluation

In each crop rotation, five rates of manure (II factor) were applied under potatoes: 0, 20, 40, 60 and 80 t• ha^{-1} every four years.

These factors were permanent in the history of the experiment.

Since 1984 (the third rotation) in the both crop rotations mineral fertilization has been set (III factor). Each crop was supplied with four rates of nitrogen fertilizers in accordance with table 4.

Crop rotation	Plant	Mineral fertilization [kg ha^{-1}]					
		N0	N1	N2	N3	P_2O_5	K_2O
A	Potatoes	0	45	90	135	54	160
	Winter wheat	0	40	80	120	54	100
	Spring barley	0	40	80	120	54	85
	Maize for silage	0	45	90	135	54	120
B	Potatoes	0	45	90	135	54	160
	Winter wheat	0	40	80	120	54	100
	Spring barley	0	30	60	90	54	85
	Clover with grass	0	40	80	120	54	115

Table 4. Mineral fertilization for crop rotation A and B

The paper presents the results of permanent experiment in which the quantity of soil organic matter was determined through 33 years. The quality of soil organic matter was evaluated on the basis of the soil samples (0-30 cm) collected after seventh rotation in the both cycles 1 and 2(Table 3). The average values for the cycles of described parameters were considered. SOM was expressed as the percentage content of organic carbon in soil.

In the experiment numerous parameters were evaluated, but for the paper purposes the follows ones were determined: organic carbon content by direct method using Analyzer C-

MAT 5500 and fractional composition of organic matter by Schnitzer method. The content of organic carbon of separated fraction was calculated as follow:

- C_d –carbon in solution after decalcification
- CHA+FA – sum of humic and fulvic acids in extracts obtained with 0,5 m NaOH
- CF – carbon of fulvic acids in solutions, following humic acids precipitation
- CHA – carbon of humic acids calculated from the difference:
- CHA = CHA+FA – CFA

The fractional composition was expressed as the percentage share of respective fraction in the total organic carbon pool (TOC).

Optical parameters of humic acids (HU) were measured in the UV-VIS, and afterwards, A_4/A_6 ratios were calculated.

The absorbance at 280, 465 and 665 nm of a solution (pH 8,3), containing at least 1 mg of in $NaHCO_3$ was measured in a UV –VIS spectrometer Perkin Elmer Lambda 20. The ratio of $A_4/A_6$was used do characterize SOM according to Kononova (1966) [16].

Statistical processing of the results was performed using Statgraphics 5 Plus package.

The data were processed by ANOVA, for each crop rotation, manure and mineral fertilization. There were proofed significant effect of crop rotation and manure application on both organic carbon content in soil and SOM quality. However, these parameters did not been affected by mineral fertilization.

The average values for treatments with different rates of manure and mineral N fertilizers describe the effect of experimental factors on soil organic carbon quantity. SOM quality was evaluated by the average values for crop rotations (A, B), and for the extreme treatments – without mineral nitrogen (N0), and the highest N rate (N3) as well as for manure, rates (1 and 5). Furthermore, the treatment with the highest mineral and manure doses was included (see explanations under table 5).

3. Results

3.1. The quantity of SOM

The analysis of variance demonstrated the importance of main effects and random effect (years of study) as well as the synergies of all the experimental factors on Corg (P-Value 0,0000 for all tests). The content of organic carbon in soil through 33 years of the experiment is presented by Figure 2. Figure 3 illustrates the impact of manure application on Corg. content in soil under cultivation of plants exhausting and enriching soil in organic matter. The results are the average for treatments with different rates of manure and mineral nitrogen fertilizers.

To understand the figure 1, it is needed to consider that for the first two four-years rotations, up to 1984, the experimental scheme included only manure application. At the beginning of the experiment in 1979, the initial organic carbon content amounted to 0, 74% (Fig. 2). After eight years, in crop rotations with clover grass mixture (B), organic carbon content oscillated around the initial value and amounted to 0,78%. Meanwhile, in crop rotation without legumes (A) Corg. value dropped to 0,72%.

In the following years (since the third rotation), in fields under plants exhausting soil with SOM (A), organic carbon quantity decreased regularly through the experiment and after 33 years dropped to 0, 61%. On the contrary, in crop rotation with clover – grass mixture, the tendency to stabilization organic carbon quantity in soil was observed with the highest value 0,79% in 1988 and the lowest one 0,72% in 2004. The disturbances in Corg. content detected through the experiment in both crop rotations could be caused by both climatic conditions and spatial field variability.

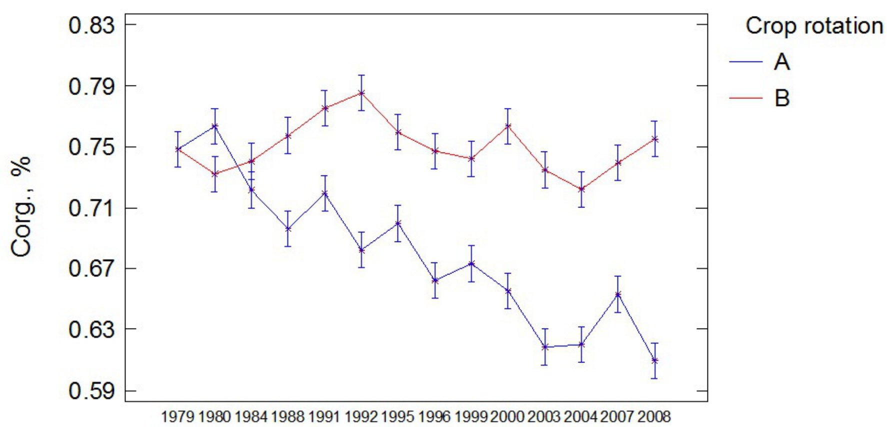

Figure 2. Effect of crop rotation on organic carbon content in soil through 1979-2008

The results illustrated by figure 3 indicated, that the effect of manure on soil organic carbon content was strongly linked to the crop rotation. In the both crop rotations, manure applicationin creased soil organic carbon. However, in crop rotations with plants exhausting soil from SOM even the highest manure rates 60 and 80 t ha^{-1} was not sufficient to secure Corg. content at the initial level (Fig. 3) over the years, what confirms the results presented by Figure 2. Meanwhile, in crop rotation with legumes, only 20 t ha^{-1} manure per hectare was required to obtain such content.

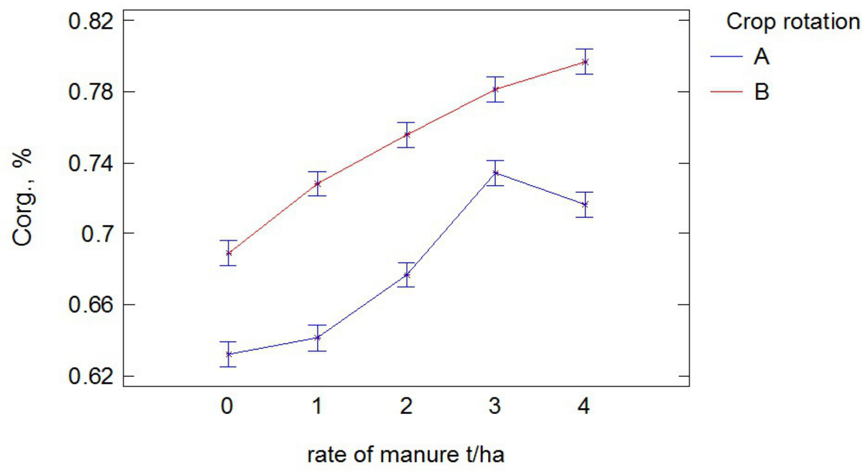

Figure 3. Effect of interaction manure application x crop rotation on soil organic carbon content

3.2. The quality of SOM

The properties of soil organic matter were evaluated on the base of its fractional composition. Table 5 shows the group composition of humus after 29 years of the experiment done by Schnitzer's method.

Combination	C_{org} %	C_d %	CHA+CFA	C_{FA}	C_{HA}	C_H	C_{HA}:C_{FA}
A1N0	0,55	0,033	47,3	20,0	27,3	52,7	1,37
A1N3	0,61	0,034	41,0	16,4	24,6	59,0	1,50
A5N0	0,74	0,039	43,3	14,9	38,4	56,7	1,91
A5N3	0,74	0,039	52,7	18,9	33,8	47,3	1,79
B1B0	0,75	0,035	48,0	20,7	27,3	52,0	1,32
B1N3	0,80	0,035	43,8	18,8	25,0	56,2	1,33
B5N0	0,78	0,042	48,7	21,8	26,9	51,3	1,23
B5N3	0,82	0,033	50,6	22,0	28,6	49,4	1,30

Table 5. Explanations: A – crop rotation with plants exhausting soil from humus B – crop rotation with plants enriching soil with humus1 – treatment without manure5 – treatment with 80 t manure ha^{-1} N0- treatment without mineral fertilizers N3 – 120 kg N ha^{-1} Fractional composition of SOM

As it has been described in Material and Methods, the results for selected treatments were listed. Constantly, the values of organic carbon content in soil samples were presented.

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Humus content and its quality in the soil depended on manure application and the choice of plant species for crop rotation. There was found that crop rotation B and manure fertilization generally increased organic C content in the soil. Crop rotation influenced the strong to quality of SOM (Fig.4).

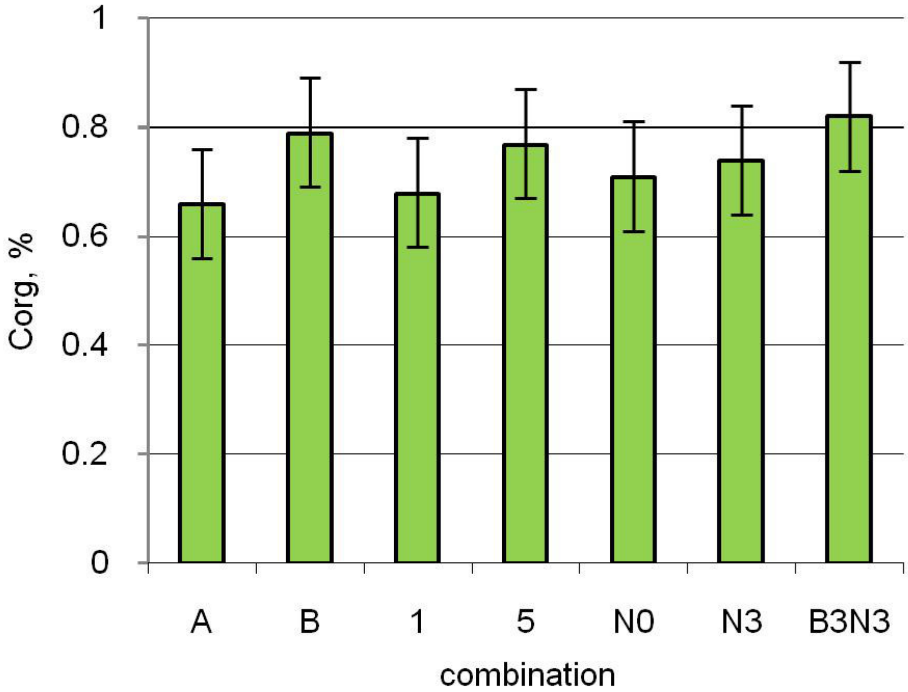

Figure 4. Effect of manure application, mineral fertilization, and crop rotation on Corg. content(explanations as under table 3). Error bars indicate standard deviation

Both manure application and selection of plants with legumes in crop rotation strongly modified the fractional composition of SOM and led to an increase of the sum of humic acids and fulvic acids in soil as well as the decrease in the amount of humins. Manure fertilization increased the ratio of humic acids in soil where as crop rotation with legumes affected a larger proportion of fulvic acids in comparison with humic acids. As a consequence, in treatments with regular, one to four years manure application, the ratio CHA:CFA was higher as compared with only mineral N fertilization. Similarly, soil in fields under clover with grasses cultivation are characterized by the lower ratio of humic acids to fulvic acids.

In the described experiment, the selection of plant species for crop rotation was recognized as crucial for the parameter. Humus under plants which enhance soil with organic matter revealed much lower values of CHA:CFA ratio as compared with the exhausting ones (Fig. 6). These differences will be discussed in the next part of the paper.

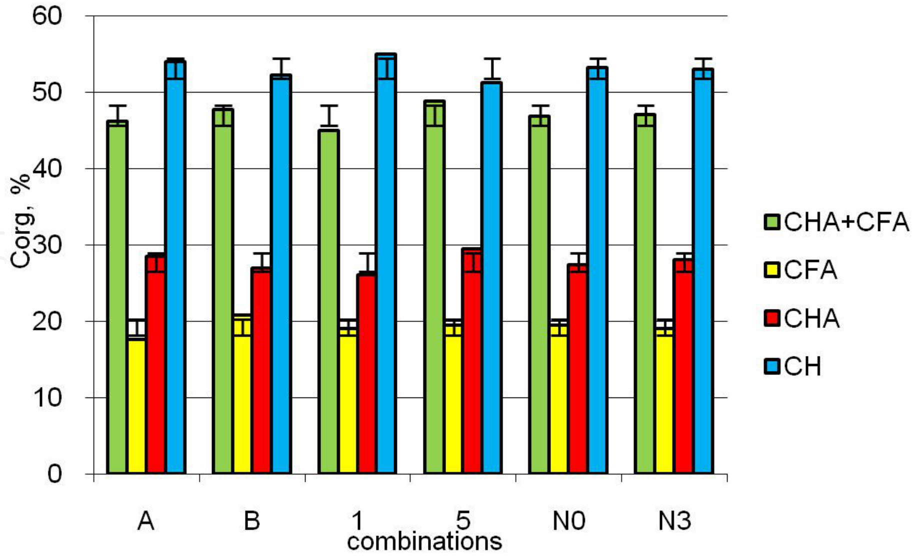

Figure 5. Fractional composition of humic substances (explanations as under table 3).

Error bars indicate standard deviation

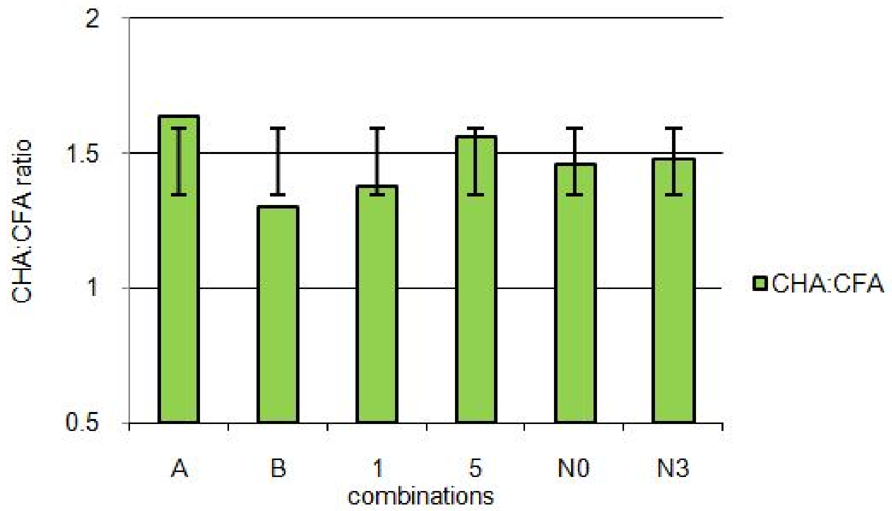

Figure 6. The ratio of humic acids to fulvic acids (CHA:CFA).Error bars indicate standard deviation

The isolated humic acids were treated by 1 mg of in $NaHCO_3$ and the liquid samples were subjected to UV - VIS radiation by the spectrometer for measurement within the range of visible and UV light. Then, the ratio of UV absorbance in different wave-lengths were analyzed. The coefficients of absorbance of humic acids were listed in table 6.

Combination	A280[1)]	A465[1)]	A665[1)]	A280/465[2)]	A280/665[2)]	A465/665[2)]
A1N0	2,16	0,401	0,0871	5,39	24,8	4,60
A1N3	2,09	0,384	0,0864	5,44	24,2	4,44
A5N0	1,85	0,325	0,0695	5,69	26,6	4,68
A5N3	2,03	0,318	0,0657	6,38	39,0	4,84
B1B0	2,08	0,346	0,0736	6,01	28,3	4,70
B1N3	1,96	0,318	0,0650	6,61	32,0	4,89
B5N0	2,04	0,306	0,30585	6,66	34,9	5,23
B5N3	2,09	0,339	0,0657	6,20	31,8	5,16

Table 6. Explanations: A – crop rotation with plants exhausting soil from humus B – crop rotation with plants enriching soil with humus1 – treatment without manure5 – treatment with 80 t manure ha^{-1} N0- treatment without mineral fertilizersN3 – 120 kg N ha^{-1} [1)]– wave-lengths (nm) [2)] – absorption ratioThe coefficients of absorbance of humic acids

The soil with manure application and mineral fertilization revealed the spectrum 280 nm in both crop rotations, which indicates the early stage of humification process characterized by high lignin content. However, there was no clear evidence in relation of fertilization and crop rotation on the intensity of the spectrum which amounted from 2,00 to 2,07 (Fig. 5).

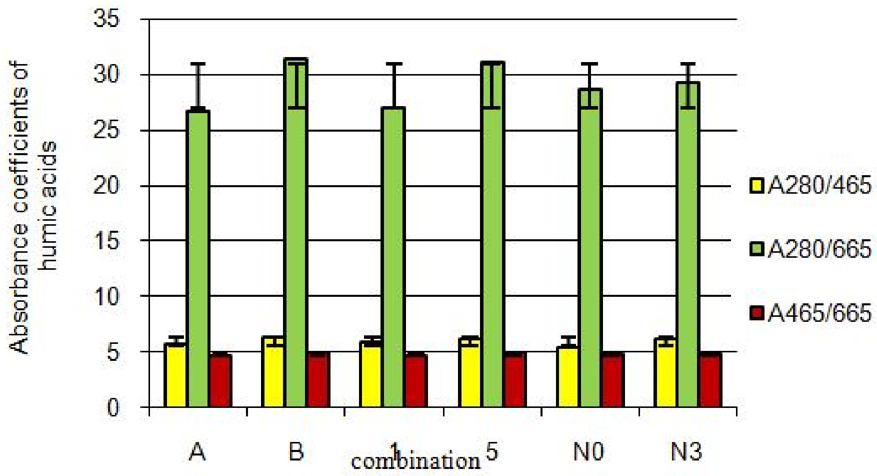

Figure 7. Absorbance coefficients of humic acids. Error bars indicate standard deviation

4. Discussion

Carbon plays a significant role both for agriculture and environment protection. The organic matter is a major component for soil formation. It considerably determines soil fertility and soil properties that are relevant to the yield. In agriculture land, both soil organic matter quantity and quality are determined by natural, agroclimatic condition as well as soil cultivation technology. The relevance of soil organic matter has been long neglected, and for decades the crop production was focused on mineral fertilization. During the last ten years in EU countries, aprogressive lost of soil organic matter has been observed. In Poland, due to simplification in crop production with prevalence of cereal monocultures as well as crop residue removal and reduced manure production intensify the process of natural SOM degradation. An of an adequate farm management relies in maintaining a sustainable balance of soil organic matter. A balance can be achieved by selection of species of cultivated plants, their share in the crop structure, and the quality of organic fertilizers. For different crop species the amount of residues left in filed varies.It can be estimated that the weight of cereals crop residues is about 3-fold greater than the roots, and legumes with grasses by up to 6-fold. Moreover, a different duration and degree of shading the soil surface and the number of tillage performed might affects the mineralization of organic compounds in humus.

The impact of all these factors on soil organic matter transformations could be accounted only in long-term perspective because of gradual character of the processes. The results of described 33 years experiment confirm the positive effect of organic fertilization and crop rotation included legumes – grass mixtures on SOM reproduction. For the research presented, the effect of crop rotation had a larger impact on organic carbon accumulation in soil that the effect of manure applications. Those results could be explained by the fact that clover mixture with grasses results ina greater amount of humus in soil compared with manure application. Moreover, carbon from manure is more prone to undergo mineralization process [20].

The reduction of Corg in soil in the following years relative to the initial value, is not significantly related with manure rates and can be explained by a rather slow depleting of the total pool of humus in agricultural soil as the effect of tillage intensity and regular removal of straw from the field [21, 22]. The results from the long term field experiment indicate that an external source of organic matter such as manure applied regularly might not assure a positive balance of SOM. The results confirmed that including legumes into crop rotation might provide an effective method to preserve the optimal level of humus.

The role of mineral nitrogen fertilization remains unclear. Some authors have described that applicaton of high amounts of mineral fertilizers might accelerate mineralization process and therefore diminish organic carbon. Other authors [23, 24] propose that prolonged application of mineral N fertilisers on lessives and brown soils may cause a decrease of carbon content in soil of about 21% compared with soils not supplied with mineral fertilizers. Numerous papers indicate that the inhance combined application of manure and mineral nitrogen fertilizers enhances the mineralization process of humus [25-27]. However, there exists a strong consensus on the literature that mineral nitrogen has beneficial effect for humus stabilization [8].

In the own research, the significant effect of mineral nitrogen fertilization did not be proven.

A distinctive/significant difference was observed in the evolution of organic carbon content in soil over time for crop rotation that included plants recognized to deplete humic substances or plants that can enrich the humic content in soil.

Soil cultivation and management practice, particular manure application and mineral fertilization, tillage systems and crop rotation induced an important discussion about humus quality. According to stability and decomposability, soil organic carbon is divided into the stable and labile forms. The stable forms are represented by the total carbon content, humic substances sum, humic acids sum and fulvic acids sum [10, 28]. The labile carbon is soluble in water, more active and undergoes short time changes in the soil. Körschnes [29] claims that this part of SOM is a useful tool to characterize the soil management practice. The objectives of own experiment was both quantitative changes in Corg content in soil and quality of humus according to Schnitzer method. That approach enables to point out the most proper soil management regarding to organic and mineral fertilization, and selection of plant species for crop rotations to obtain the positive balance of SOM and soil fertility measured by humus quality [29-31]. SOM quality is determined not by the absolute quantity of humus acids but its percentage share in humus. Humic and fulvic acids content underlies the calculation HA:FA ratio. The ratio describes humus quality and its stability as well. It is commonly accepted that fertile soils are characterized by the ratio HA:FA >1. Soils fertilized with composts, slurry and manure display higher HA:FA ratio than soils supplied with mineral fertilizers [32]. The research presented provides similar results. Furthermore, the strong impact of crop rotations on the ratio was found. Humus of the soil under clover with grasses mixture (crop rotation B) revealed lowest value of the ratio than with maize for silage (crop rotation A) which amounted to 1.30 and 1.64 respectively. The literature reports that the lowest ratio of humic acids to fulvic acids in soil under legumes cultivation, comes off from the great carbon amount left by these plants, which does not impact on the ratio [8]. Besides, legumes affect the rise of fulvic acids fraction and the lower HA:FA ratio as the result.

Fractional composition of humus was modified both by crop rotation and manure application. Legumes left a great amount of residues abundant with carbon and nitrogen - the source energy for soil microorganisms, which promote the process of humus transformation with the prevalence of mineralization one [32-35]. Hence, soils under legumes cultivation are characterized by the lower HA:FA ratio. Nevertheless, the further detailed analyzes of UV-VIS proved the good quality of soil under legumes. It demonstrates that fluorescence, although poorly utilized for humic acids research, is a powerful tool to contribute to the knowledge and the action of the organic matter in soil.

UV-VIS absorption technique is applied for a long time for the characterization of humic substances. It is commonly accepted that absorbance at 280 nm is an indicative of lignin content where as absorbance at 465 nmcan be related to organic matter in early stages of decomposition, and absorbance at 665 nm with advanced humification process of SOM. The data presented in the literature indicate [14, 17, 35] that for a UV- VIS spectrum of "young" humic acids the maximum signal at 280 nm will disappear with advanced humification process. Such spectrum is observed in soils fertilized with slurry and mineral fertilizers. For the research presented, the soil treated with manure revealed a maximum at 280 nm for both crop rotations. However, results on signal intensity at 280 were no conclusive for fertilization and crop rotation.

Higher values of the A465/665 absorbance ratio were recorded in the soil with crop rotation B and in the soil fertilized with manure, especially the highest rates. It is a clear evidence that there were "young" humic acids of a lower condensation level of aromatic structures that predominate in the structure of humic acids as compared with HA in the advanced humification process [14, 18, 36].

Manure applications as compared to mineral nitrogen fertilization increases the content of lignins in the particles of humic acids (higher values of the A280/465 absorbance ratio) (Fig. 5). Manure promoted a formulation of humus with greater share of aliphatic structure (lower absorption values) and higher (higher values of A465/665).

Maintaining or increasing soil organic matter (SOM) is justified both from an agronomic and a climatic perspective because it affects the capacity of the soil to sustain crop growth, is an important factor in decreasing soil compaction and erosion, and is also a source and possible sink of atmospheric CO_2-C [37]. Understanding the processes that control SOM dynamics is the key to SOM management. Land use and agricultural management practices such as crop rotation, soil tillage and organic amendments can affect SOM by influencing both the quantity and quality of crop residues that are returned to the soil; they also influence the rate of decomposition of added residues and native SOM [38-39].

5. Conclusions

1. The results of 32 – years of the field experiment show that the most important factor which stabilizes organic carbon content in agricultural soils is crop rotation with legumes. This effect has not been obtained even by systematic application of the very high rates of manure.
2. Because of intensified soil microbial activity, the ratio of humic acids to fulvic acids was lower in soil under legumes cultivations as compared with manure application. The further UV – VIS spectral analysis of humic acids indicated a high quality of humus of soils under clover with grasses mixture.
3. Mineral fertilization has not modified both soil organic carbon content and humus quality.

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