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Long-Term Mineral Fertilization and Soil Fertility

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1. Introduction

Long-term experiments are very important in studying the changes of soil fertility and environmental conditions as well as in analyzing the stability and quality of crop production. Such experiments give us more information how to use the good agronomic practices and how to protect the nature. Probably the oldest still-running arable crop fertilizer experiment is the Broadbalk Experiment established by John B. Lawes in Rothamsted (UK) in 1843 (Goulding et al., 2000). Thanks to this experiment many other long-term fertilizer experiments were established worldwide (Sims, 2006; Khan et al., 2007; Takahashi&Anwar, 2007; Kunzova&Hejcman, 2009).

In Bulgaria also have investigations on such long-term fertilizer trails (Koteva, 2010; Panayotova, 2005; Nankova et al., 1994 & 2005; Nankova, 2010).

Dobrudzha Agricultural Institute-General Toshevo is situated in North-Eastern part of Bulgaria on black earth zone (Picture 1). The main soil type is chernozem (Haplic Chernozems WRBSS, 2006).

The aim of this investigation was to follow the effect of the long-term agronomy practices and especially fertilization on the nutrition regime of slightly leached chernozem soil in the region of South Dobrudzha after 40 years mineral fertilization with different norm and combination between nitrogen, phosphorus and potassium.

A long-term fertilizer experiment , which was established in 1967 is still running. In two field crop rotation (wheat-maize) four nitrogen and phosphorus and three potassium norms were tested – 0, 60, 120, 180 and 0, 60, 120 kg/ha respectively. The experiment was designed according to the method of the "net square", applying the full version of the design in four replications. The experiment was designed by the method of the "net square", applying the full version of the design (4 x 4 x 3 = 48) in four replications. On the 40th year from the beginning of the trial (2007) after wheat harvest, soil samples were taken every 20 cm down the soil profile till depth 400 cm. A motor-driven portable soil sampler was used (Iliev&Nankova, 1994; Iliev, 2000). The changes of some agrochemical characteristics were determined in selected variants with high average 40th year productivity.

Picture 1. Position of Dobrudzha Agriculture Institute on Bulgaria map (43° 40′ northen latitude and 28° 10′ eastern longitude)

The soil acidity forms were determined by Ganev&Arsova (1980).

The potential nitrogen-supplying ability of soil was determined through incubation under constant temperature of 30° C at 60 % humidity from its total moisture absorption capacity in order to develop optimal conditions for nitrification. Incubation was done in thermostate to investigate its dynamics at the 14th, 28th and 56th day. The samples were analyzed to determine the amount of nitrate nitrogen in 1 % K_2SO_4 extract. The ability of NO_3-N to form intensive yellow coloration when interacting with disulphurphenoloc acid [$C_6H_3OH(HSO_3)_2$] in alkali media was used.

Carbon contend was valuated using the Tyurin modification (oxidizing with $K_2Cr_2O_7/H_2SO_4$ solution in thermostate at 125^{0}C, 45 min, at presence of Ag_2SO_4 and titration with $(NH_4)_2SO_4.FeSO_4.6\ H_2O$ (Kononova&Belchikova,1961; Spiege at al, 2007; Hegymegi at al, 2007). Composition of soil organic matter was determined by Konnova (1963) and Filcheva&Tsadilas (2002).

Data were analysed with Excel and SPSS 16.0 (2007) and means separated by the Waller-Duncan test (P<0,05).

2. Influence of long-term mineral fertilization on some agrochemical characteristics of slightly leached chernozems (Haplic Chernozems)

One of the main degenerative processes in soil is the so called acidification. Various acid complexes are formed in soil as a result from soil formation processes on the one hand (erosion, humification, leaching, podzolization), and on the other – as a result from the activity of micro organisms and plants. Soils also possess buffer systems to counteract the acidification, which differ by their capacity.

2.1 Changes in soil acidity forms

2.1.1 Soil acidity forms for the 0-400 cm profile

The soil acidity forms, averaged for the investigated depth of the 0-400 cm profile, were significantly affected by the type of fertilizer combination. The independent effect of the factor mineral fertilization was higher on exchangeable Al^{3+}, Ca^{2+} and the sum of Ca^{2+} and Mg^{2+}, and significantly lower - on the values of residual hydrolytic acidity and the rate of alkali saturation. The depth of the investigated profile was the factor with decisive effect on all forms of soil acidity. Its effect on the pH values, the residual hydrolytic acidity and the alkali saturation degree was over 90 %. Significantly lower was its influence on the exchangeable Al^{3+} (22.5%) and the sum of exchangeable Ca and Mg (45.8%).

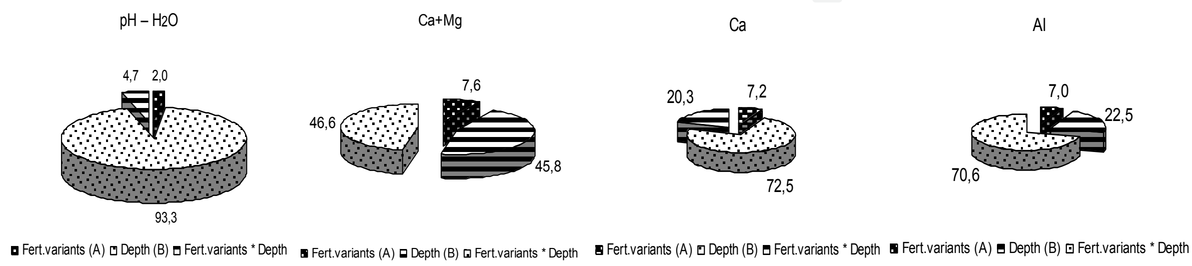

Fig. 1. Power of factors influence

In spite of the maximum degree of significance of the effect of mineral fertilization on the forms of soil acidity, the amplitude of variation of the separate indices was not so well expressed as in the separate soil layers up to 400 cm down the soil profile. Averaged for the fertilization variants, pH varied from 6.35 (10-20 cm) to 8.53 (260 – 300 cm). Soil reaction increased down the soil profile and at the 4th meter there was well expressed correlation between the soil layers forming it. It, however, showed similarities to layers 160-180, 180-200 and 200-220 cm. The layers from 220 to 300 cm possessed higher pH values in comparison to the layers of the 4th meter.

The amount of exchangeable Ca^{2+} showed a gradual tendency toward decreasing down the depth profile. Amplitude of variation was from 28.49 $cmol_c kg^{-1}$ (60-80 cm) to 18.79 $cmol_c kg^{-1}$ (380-400 cm). The surface layers 0-10 and 10-20 cm had lower content of exchangeable Ca^{2+} in comparison to the layers under them up to depth of 100 cm, being more similar to the amounts found in the 2nd meter. Highest amounts were detected in layers 60-80 cm and 80-100 cm.

The amount of exchangeable Mg^{2+} had a clear tendency toward increasing down the soil profile, being highest in the 340-360 cm layer (8.10 $cmol_c kg^{-1}$). In the trial field, layers 80-100, 120-140 and 60-80 cm had lowest content of exchangeable Mg^{2+} – about 1-2 $cmol_c kg^{-1}$. The surface layers within the 1st meter were comparatively richer in it, but their content considerably conceded to the content in the deeper layers of the 3rd and 4th meter.

The sum of the two exchangeable cations down the profile varied from 25.38 $cmol_c kg^{-1}$ (120-140 cm) to 30.51 $cmol_c kg^{-1}$ (60-80 cm). The surface layers (0-10 cm and 10-20 cm) had lower sorption capacity, ∑Ca+Mg and degree of saturation with bases than the 0-20 cm layer according to the trial beginnig. According to Nankova (2005, Personal Communication) at the start of this long-term experiment the values of these parameters were 34,44 $cmol_c kg^{-1}$, 30,80 $cmol_c kg^{-1}$ and 91,2% respectively. Further down the profile the sorption capacity decreased. What is very impressive is that it significantly increased in the 4th meter regardless of the occurrence of Ha in the 360-380 and 380-400 cm layers. The main reason for this is the higher amount of exchangeable Mg^{2+} in the 4th meter, which makes it very distinctive from the layers above it.

The degree of saturation with bases was lowest in the surface layers 0-10, 10-20 and 20-40 cm due to the intensive anthropogenic activity on the one hand, and on the other – due to the presence of plants. From the 40-60 cm layer the values of this index increased. In the entire 2nd meter the degree of saturation with bases was more than 99 %, and in the third meter it was 100 %. This value remained the same in the upper part of the 4th meter but in the layers 360-380 and 380-400 cm decreased slightly and was closer to the values registered in the 2nd meter. The reason for this is the occurrence of residual hydrolytic acid in the lower part of the 4th meter.

The separate meters up to depth 400 cm, as well as the sub-layers (horizons) at each meter (every 20 cm) affected to a maximum degree of significance the investigated indices characterizing the soil acidity forms in the investigated fertilization variants. The comparison of the results for the indices characterizing soil acidity revealed clear differentiation by each meter down the investigated soil profile.

Depth, cm	pH/ KCl	pH/H_2O	$T_{8.2,}$ Sorption capacity	Exchangeable cations					Degree of saturation with bases, V%
				$H_{8.2}$	Al^{3+}	Ca^{2+}	Mg^{2+}	∑Ca+Mg	
			In $cmol_ckg^{-1}$. soil						
0-10	5,54 b	6,46 b	33,21 p	5,65 k	,17 b	23,80 k	3,60 h	27,40 h	82,99 b
10-20	5,38 a	6,35 a	33,06 o	5,90 l	,26 c	23,27 i	3,62 h	26,89 f	82,17 a
20-40	5,72 c	6,74 c	33,06 o	4,65 j	,00 a	25,09 m	3,33 g	28,42 l	85,93 c
40-60	6,45 d	7,48 d	32,83 n	2,88 i	,00 a	27,44 n	2,52 e	29,95 n	91,17 d
60-80	6,80 e	7,89 e	32,13 m	1,62 h	,00 a	28,49 p	2,02 c	30,51 o	94,91 e
80-100	7,15 f	8,21 f	30,83 l	,97 g	,00 a	28,28 o	1,58 a	29,86 m	96,89 f
100-120	7,35 g	8,30 g	28,37 k	,47 f	,00 a	24,97 l	2,94 f	27,90 j	98,38 g
120-140	7,43 jk	8,34 h	25,56 a	,19 e	,00 a	23,64 j	1,74 b	25,38 a	99,29 h
140-160	7,41 i	8,40 i	26,00 d	,09 d	,00 a	23,67 j	2,25 d	25,91 c	99,67 i
160-180	7,39 h	8,48 jk	26,54 f	,03 b	,00 a	23,58 j	2,94 f	26,51 e	99,91 k
180-200	7,41 i	8,51 klm	26,53 f	,04 b	,00 a	23,08 h	3,41 g	26,49 e	99,86 k
200-220	7,42 ij	8,47 j	26,97 g	,00 a	,00 a	23,11 h	3,86 i	26,97 f	100,00 l
220-240	7,43 k	8,52 m	26,51 f	,00 a	,00 a	22,05 g	4,46 k	26,51 e	100,00 l
240-260	7,49 m	8,52 lm	25,88 c	,00 a	,00 a	21,62 f	4,25 j	25,88 c	100,00 l
260-280	7,47 l	8,53 m	25,88 c	,00 a	,00 a	21,55 f	4,32 j	25,88 c	100,00 l
280-300	7,47 l	8,53 m	25,70 b	,00 a	,00 a	19,88 b	5,82 l	25,70 b	100,00 l
300-320	7,49 m	8,48 j	27,24 h	,00 a	,00 a	21,09 e	6,15 m	27,24 g	100,00 l
320-340	7,49 m	8,48 jk	28,25 j	,00 a	,00 a	20,79 d	7,46 n	28,25 k	100,00 l
340-360	7,52 n	8,49 jkl	28,33 jk	,00 a	,00 a	20,23 c	8,10 p	28,33 k	100,00 l
360-380	7,51 n	8,49 jkl	27,75 i	,06 c	,00 a	19,98 b	7,71 o	27,69 i	99,79 j
380-400	7,53 o	8,49 jkl	26,36 e	,06 c	,00 a	18,79 a	7,51 n	26,30 d	99,79 j

Table 1. Sorption capacity ($T_{8.2}$), exchangeable cations and degree of saturation with bases down the soil profile

Depth, cm	pH/ KCl	pH/ H_2O	$T_{8.2}$	Exchangeable cations					Degree of saturation with bases
				$H_{8.2}$	Al^{3+}	Ca^{2+}	Mg^{2+}	∑Ca+Mg	
			In $cmol_c kg^{-1}$. soil						
0-100	6,32 a	7,35 a	32,40 d	3,18 d	,043 b	26,57 d	2,61 a	29,186 d	90,30 a
100-200	7,40 b	8,41 b	26,60 b	,16 c	,000 a	23,79 c	2,65 a	26,44 b	99,42 b
200-300	7,46c	8,51 d	26,19 a	,00 a	,000 a	21,64 b	4,54 b	26,19 a	100,00 d
300-400	7,51 d	8,48 c	27,59 c	,025 b	,00 a	20,18 a	7,39 c	27,56 c	99,91 c

Table 2. Sorption capacity ($T_{8.2}$), exchangeable cations and degree of saturation with bases by meter down the soil profile

Averaged for the investigated fertilization variants, the 1st meter had lowest pH values, exchangeable Mg^{2+} and degree of saturation with bases. This meter, at the end of the 40-year period of investigation, showed harmful exchangeable acidity untypical for the natural status of this soil type. The first meter was also characterized by significantly higher content of residual hydrolytic acidity and higher sorption capacity in comparison to the other depths down the profile. In the 2nd and 3rd meter, with the exception of pH and the degree of saturation with bases, all other indices decreased their values. The third meter was characterized with complete absence of residual hydrolytic acidity, 100 % saturation with bases and increased content of exchangeable Mg^{2+} - with 73.9 % more above the 1st meter and with 71.2 % more above what has been established in the 2nd meter. What was typical for the separate layers of the 4th meter, besides the visually distinct coloration of these layers, was once again the occurrence of residual hydrolytic acidity, comparatively high increase of the content of exchangeable Mg^{2+} in comparison to the 3rd meter (62.6 %). Averaged for this depth, a higher sum of exchangeable bases was determined in the trial field in comparison to the 2nd and the 3rd meter, as well as higher sorption capacity of soil.

In spite of the average data for 400 cm, 40 years mineral fertilization caused a big difference on the forms of soil acidity accorfing to kind of fertilizer variant. The differentiation between variants of fertilization is very well expressed (Table 3). Soil reaction varied in narrow limits, but in spite of this it was established decreasing of pH in variants $N_{180}P_{60}K_{60}$ и $N_{60}P_{180}K_0$ according to the control variant. By the Waller-Duncan test there were established a very well expressed differences in all soil acidity forms, as well as in degree of saturation.

The lowest values of residual hydrolytic acidity ($H_{8,2}$) were registered in the variant with independent fertilization with 180 kg P_2O_5/ha (0.87 $cmol_c kg^{-1}$ soil). Residual hydrolytic acidity is one of the forms strongly affected by the long-term mineral fertilization, especially in the variant with $N_{180}P_{60}K_{60}$ (1.36 $cmol_c kg^{-1}$ soil). High amplitude of variation was determined for residual hydrolytic acidity down the soil profile: from its complete lack to 5.90 $cmol_c kg^{-1}$ soil. As was shown the highest values were established in the surface layers, 0-10 and 10-20 cm, which are most influenced by the agronomy practices fertilization and tillage.

The strongest evidence for the high effect of the long-term mineral fertilization with various norms and ratios on the agrochemical condition of the slightly leached chernozem soil in the trial field was the occurrence of exchangeable Al^{3+} in the soil absorption complex. It was detected in the surface layers (0-10 and 10-20 cm) in the variants $N_{180}P_0K_0$ and $N_{180}P_{60}K_{60}$. It was not present in the soil absorption complex further down the profile.

Fertilizer variants	pH/ KCl	pH/ H_2O	$T_{8.2}$	Exchangeable					Degree of saturation with bases – V %
				$H_{8.2}$	Al	Ca	Mg	Ca+Mg	
			cmol$_c$kg^{-1}. почва						
$N_0P_0K_0$	7,12 c	8,14 d	28,55 d	1,03 c	,00 a	24,60 f	2,92 a	27,52 d	96,83 f
$N_{60}P_0K_0$	7,18 f	8,13 d	28,71 e	1,13 f	,00 a	23,16 d	4,41 d	27,58 e	96,50 c
$N_{120}P_0K_0$	7,13 d	8,15 d	28,96 g	,89 b	,00 a	23,79 e	4,29 c	28,07 h	97,30 g
$N_{180}P_0K_0$	7,13 d	8,23 e	27,45 a	1,11 e	,07 b	21,63 a	4,64 e	26,27 a	96,59 d
$N_0P_{180}K_0$	7,15 e	8,21 e	28,72 e	,87 a	,00 a	23,19 d	4,65e	27,84 g	97,39 h
$N_{60}P_{180}K_0$	6,98 a	7,96 b	27,94 b	1,16 g	,00 a	22,92 c	3,87 b	26,78 b	96,41 b
$N_{120}P_{120}K_{120}$	7,04 b	8,06 c	28,79 f	1,05 d	,00 a	22,88 c	4,86 f	27,74 f	96,69 e
$N_{180}P_{60}K_{60}$	6,98 a	7,93 a	28,30 c	1,36 h	,10 c	22,36 b	4,48 d	26,84 c	95,89 a

Table 3. Sorption capacity ($T_{8.2}$), exchangeable cations and degree of saturation with bases according to variants of fertilization, average for 0-400 cm depth

The variant with independent nitrogen fertilization with 180 kg/ha has the lowest ∑ Ca+Mg and the lowest value of sorption capacity. Intensive mineral fertilization with $N_{180}P_{60}K_{60}$ caused decreasing of degree of saturation with bases.

2.1.2 Compare the changes of soil acidity forms after 30th and 40th years mineral fertilization

For the first time in Bulgaria in such long-term trial we compared the obtained results for the individual indices which characterize the forms of soil acidity for the end of the 30th and the end of the 40th year since the initiation of the experiment in some of the variants to 60 cm depth.

At the end of the 40th year, the tendency towards lower values of pH and sorption capacity of soil became more prominent to various degrees according to the type of fertilization variant . This tendency was most evident in the variant with $N_{120}P_{120}K_{120}$, where a decrease with 17.2 % according to the end of the 30th year was determined.

A serious change was observed also towards decrease of the values of acidity on the strongly acid positions (T_{CA}) of the soil adsorbent with the increase of the duration of mineral fertilization in the above variants, and respective significant increase of this acidity, but this time on the slightly acid positions of the soil adsorbent. The indicated change led also to decrease of the rate of alkali saturation. The decrease varied from 4.4% ($N_{120}P_0K_0$) to 8.1% ($N_{120}P_{120}K_{120}$).

Fertilizer variants	pH/H_2O		$T_{8.2}$ Sorption capacity		T_{CA} Strongly acid positions		T_A Slightly acid positions		Degree of saturation with bases	
	30th	40th	30th	40th	30th	40th	30th	40th	30th	40th
$N_0P_0K_0$	7,60 c	6,85 a	34,87 b	32,93 b	32,33 b	23,92 b	2,53 ab	9,01 b	92,63 b	85,65 a
$N_{120}P_0K_0$	7,48 b	7,09 c	35,17 b	33,15 c	32,55 b	24,11 b	2,62 b	9,03 b	92,59 b	88,55 c
$N_{180}P_0K_0$	7,14 a	6,99 b	32,74 a	32,92 b	29,57 a	24,83 c	3,18 c	8,09 a	91,09 a	86,84 b
$N_{120}P_{120}K_{120}$	7,44 b	6,84 a	38,32 c	31,71 a	35,87 c	22,23 a	2,45 a	9,48 c	93,15 c	85,65 a

Table 4. Comparison of the soil acidity forms at the end of the 30th and at the end of the 40th year from the initiation of the trial according to the fertilization variant applied

Furthermore, during the last decade the balanced treatment with NPK at norm 120 kg/ha was the variant with lower values of $T_{8,2}$ and T_{CA}, while at the end of the 30th year of this trial the values of the above indices were higher than the values of the check variant and the values of the variants with independent nitrogen fertilization with increasing norms. The negative changes in the absolute values of these indices occurred also when determining the percent of acidity on the highly acid positions of the soil adsorbent (Fig. 2).

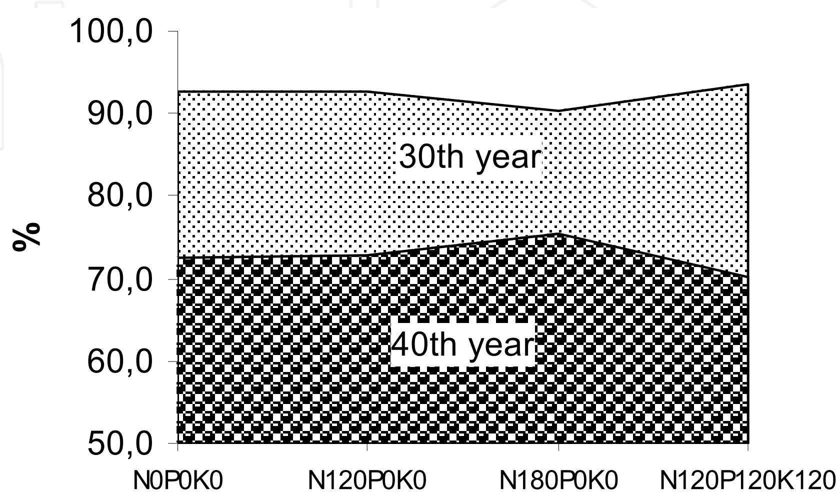

Fig. 2. Acidity on the highly acid positions of the soil adsorbent as percent of $T_{8.2}$ according to the fertilization variant at the end of the 30th and the 40th year of the trial.

The changes caused by the long-term agricultural usage of the land concerned also changeable Ca and Mg and their respective sum (Table 5). During the last investigated period changeable Ca^{2+} decreased in all tested variants, most strongly in the variants treated annually with $N_{120}P_{120}K_{120}$. The check variant ($N_0P_0K_0$), as well the variants with independent nitrogen fertilization, were less affected by this process. The amount of exchangeable Ca^{2+} at the end of the 40th year averaged for the investigated variants was 88.76 % from the amount at the end of the 30th year. A tendency was found towards lower amounts of exchangeable Mg^{2+} in the check variant and the variants with independent nitrogen fertilization, and towards significant increase in the variant with systematic balanced introduction of the main macro elements ($N_{120}P_{120}K_{120}$). At the end of the 30th year the investigated variants were characterized with a mean content of exchangeable Mg^{2+} of 3.66 $cmol_c kg^{-1}$ soil, and at the end of the 40th year – of 3,05 $cmol_c kg^{-1}$ soil. The comparison of the results for ∑ Ca+Mg revealed their decrease with averagely 12 % according to the data from the end of the 30th year, the decrease being greatest in the variant $N_{120}P_{120}K_{120}$.

Fertilizer variants	Exchangeable cations							
	Ha		Ca^{2+}		Mg^{2+}		∑Ca+Mg	
	30th	40th	30th	40th	30th	40th	30th	40th
$N_0P_0K_0$	2,57 a	4,73 d	28,63 b	25,12 b	3,63 b	3,00 ab	32,27 b	28,12 b
$N_{120}P_0K_0$	2,58 a	3,80 a	28,23 b	26,98 c	4,33 c	2,38 a	32,57c	29,36 d
$N_{180}P_0K_0$	2,60 a	4,33 b	25,82 a	25,49 b	3,78 b	3,11 bc	29,60 a	28,60 c
$N_{120}P_{120}K_{120}$	2,92 b	4,56 c	31,17 c	23,43 a	2,88 a	3,72 c	34,05 d	27,15 a

Table 5. Comparison of the changes in the exchangeable cations at the end of the 30th year and at the end of the 40th year from the trial depending on the type of the fertilization variant

At the end of the 40th year the acidity on the highly acid positions averaged for depth 0-60 cm was 23.77 $cmol_c kg^{-1}$ soil, compared to 32.58 $cmol_c kg^{-1}$ soil at the end of the 30th year, i.e. there was a decrease with 27.04 % (Table 5). pH variations affected most strongly the 20-40 cm layer. The established negative tendencies affected the 40-60 cm layer as well, where a significant decrease of the sorption capacity of soil was determined: with 9.6 % according to the values at the end of the 30th year.

At the same time the acidity on the slightly acid positions strongly increased in all three layers, the mean increase being almost three times higher, and affected mostly the 40-60 cm layer. These results also concern the rate of alkali saturation, which, too, demonstrated a tendency towards decrease. The decrease was highest in the surface 0-20 cm layer (8.1 %), in the 20-40 cm layer (6.5 %) and in the 40-60 cm layer (4.1 %).

Soil depth, cm	pH/H_2O		$T_{8.2}$ Sorption capacity		T_{CA} Strongly acid positions		T_A Slightly acid positions		Degree of saturation with bases	
	30th	40th	30th	40th	30th	40th	30th	40th	30th	40th
0-20	6,99 a	6,42 a	34,77 a	32,69 b	31,21 a	22,89 a	3,56 c	9,80 c	89,49 a	82,29 a
20-40	7,44 b	6,86 b	35,24 b	32,96 c	32,39 b	24,01 b	2,85 b	8,96 b	92,51 b	86,49 b
40-60	7,81 c	7,55 c	35,81 c	32,38 a	34,14 c	24,42 c	1,67 a	7,96 a	95,11 c	91,25 c

Table 6. Comparison of the soil acidity forms at the end of the 30th year and at the end of the 40th year of the trial depending on the depth of the layer

The changes which occurred down the investigated profile confirmed the established tendencies towards change in the values of the exchangeable cations during the respective periods of investigation depending on fertilization. The increase of the values of residual hydrolytic acidity affected most the surface 0-20 cm layer (Table 6). This layer was characterized with highest decrease of the changeable Ca^{2+} values, the amount of exchangeable Mg^{2+} remaining practically the same. Within both periods of analysis the sum of exchangeable alkali increased down the profile due to the exchangeable Ca^{2+}.

Soil depth, cm	Exchangeable cations							
	Ha		Ca		Mg		Ca+Mg	
	30th	40th	30th	40th	30th	40th	30th	40th
0-20	3,65 c	5,79 c	27,25 a	22,96 a	3,76 b	3,90 c	31,01 a	26,86 a
20-40	2,62 b	4,45 b	27,46 a	25,52 b	4,61 c	2,99 b	32,08 b	28,52 b
40-60	1,74 a	2,83 a	30,68 b	27,28 c	2,60 a	2,26 a	33,28 c	29,54 c

Table 7. Comparison of changes in the exchangeable cations between the 30th and the 40th year from the trial depending on the depth of soil layer

2.2 Changes of the soil mineralization ability after 40-years mineral fertilization

Transportation, redistribution and transformation of nitrogen down the soil profile was affected by a number of factors such as the structure of soil units, aeration, macro pores, composition, amount and depth of post harvest residue incorporation, mineral fertilization and nitrogen norm, mineralization of organic substance, leaching, productive moisture, etc (Goldbi et al., 1995; Karlen et al., 1998). The size of the nitrogen norm is significant for agricultural production under moist, semi-dry and dry conditions to obtain acceptable balance between economic and non-economic part of the produce and avoid possible losses (Cantero-Martinez et al.,1995). It is well known that the availability of the nitrogen from the mineral fertilizers depends strongly on the type of the nitrogen source, the soil type, the crop, the fertilization norm, etc. Many farmers tend to apply higher nitrogen norms to ensure higher yields (Franzluebbers et al., 1999). This in many cases is not necessary due to changes in the distribution of the nitrogen in the surface of the soil profile and its improved mobility (Rice et al., 1986).

The ability of soil to nitrify nitrogen under optimal conditions was significantly affected by the mineral fertilization and the investigated layer up to depth 400 cm (Table 7). During all three investigated periods of increasing incubation, this effect was significant to a maximum degree both under the independent influence of the investigated factors and under their interaction.

Source	Dependent Variable	df	Mean Square	F	Sig.
Fertilizer variants (A)	14 days	7	1201,997	1071,012	,000
	28 days	7	895,323	644,004	,000
	56 days	7	1760,257	808,230	,000
Soil depth (B)	14 days	20	3566,615	3177,951	,000
	28 days	20	9128,237	6565,923	,000
	56 days	20	14022,587	6438,532	,000
A x B	14 days	140	84,304	75,117	,000
	28 days	140	63,912	45,972	,000
	56 days	140	113,244	51,997	,000

Table 8. Variance analysis of the mineralization ability during a 40-year period of investigation

The depth of the soil layer was the factor with higher effect on the values of the soil's mineralization ability in comparison to mineral fertilization during all three incubation periods (Figure 1). The longer the period of incubation was, the higher its effect, reaching a maximum at 28-day incubation. Regardless of a slight decrease in the effect of this factor at 56-day incubation, the longer incubation had higher effect on the obtained results in comparison to 14-day incubation. The effect of mineral fertilization was lowest in the second incubation period and slightly increased in the third incubation period. The long-term mineral fertilization affected the amount of the established NO_3-N to a highest degree at 14-day incubation. The same was valid for the interaction between the two factors.

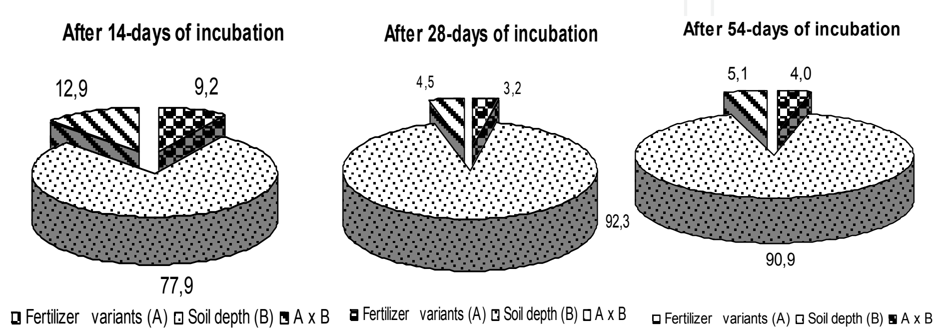

Fig. 3. Effect of factors according to the period of incubation, %

The distribution of the amount of nitrified nitrogen averaged for the periods of incubation by meters down the soil profile showed interesting results (Table 8). The soil layers of the 1st meter had highest potential nitrogen-supplying capacity. The layers forming the 2nd and the 3rd meter (loess horizon) had lowest nitrification capacity regardless of the favorable conditions for this process. The Waller-Duncan test did not reveal differences between them. It, however, differentiated the results obtained for the content of NO_3-N averaged for the 4th meter in a separate group after what was established in the 1st meter.

Meters	Value	Group
2	3,59	a
3	3,74	a
4	5,08	b
1	32,92	c

Table 9. NO_3-N content by meters down the soil profile (mg NO_3-N/1000 g soil)

The effect of mineral fertilization of the different variants averaged for depth 0-400 cm and the incubation periods was strongly expressed depending on the norms and ratios between the three macro elements (Table 9).

Fertilizer variants	Value	Group
$N_0P_0K_0$	5,95	a
$N_{120}P_{120}K_{120}$	8,64	b
$N_0P_{180}K_0$	9,17	c
$N_{60}P_0K_0$	10,28	d
$N_{60}P_{180}K_0$	10,71	d
$N_{120}P_0K_0$	11,36	e
$N_{180}P_0K_0$	12,93	f
$N_{180}P_{60}K_{60}$	21,63	g

Table 10. Mean content of NO_3-N according to the type of fertilization variant (mg NO_3-N/1000 g soil)

The check variant ($N_0P_0K_0$) reflected the natural fertility of *Haplic Chernozems* in the trial field after its long-term cultivation. The check variant had lowest content of NO_3-N after incubation among all tested variants. The fertilization variants were well differentiated on the basis of the total amount of NO_3-N after incubation. The independent nitrogen fertilization with increasing norms was accompanied with proportional increase of the amount of nitrified nitrogen, the values of which fell within separate groups of higher orders, compared one to another.

When combining nitrogen with phosphorus and potassium depending on the norms and ratios between the three elements, the 4-m soil layer had variable capacity to supply nitrates as a result from incubation. Highest amounts of this inorganic nitrogen form were found after systematic application of $N_{180}P_{60}K_{60}$ – 21.63 mg NO_3-N/1000 g soil. The systematic application of $N_{120}P_{120}K_{120}$ for a period of 40 years showed lowest amounts of nitrified nitrogen following the check variant. Averaged for the 4-m soil profile, they were lower than the amounts after independent application of the same nitrogen norm. The main reason for this was that after this type of fertilization the highest yields from wheat were obtained, averaged for the 40-year period of investigation, which, on its part, was an indication for their uptake and respective realization into cash crop. The results with regard to the nitrification capacity from the analysis of this fertilization variant revealed considerable similarities to that of the check variant. The comparatively low amounts of nitrified nitrogen after systematic fertilization with $N_{120}P_{120}K_{120}$, combined with high agronomy effect, showed that this fertilization combination can not lead to accumulation of inorganic nitrogen in soil (in nitrate form) down the soil profile.

The incubation periods of soil under conditions favorable for the nitrification process also significantly affected the values of nitrified nitrogen (Table 10). With the longer incubation periods, the mean total amount of nitrified nitrogen increased with increasing the days of incubation. This lead to clear differentiation of the incubation periods and to formation of the results into separate groups.

Days of incubation	Value	Group
14	7,2081	a
28	11,0113	b
56	15,7811	c

Table 11. Mean content of NO_3-N according to the incubation period (mg NO_3-N/1000 g soil)

2.3 Changes of the soil organic matter after long-term mineral fertilization

2.3.1 Carbon concentration along the soil profile to 400 cm depth

Systematic mineral fertilization carried out for 40 years in two-field crop rotation (wheat – maize) affected the content of Ctotal deep down the profile of the slightly leached chernozem soil. Annual fertilization with N180P60K60 during 40 years contributed most for the increase of its content at average depth 0-400 cm. Independent nitrogen fertilization with increasing norms, especially with 120 and 180 kg N/ha, had low effect on the content of Ctotal, averaged for depth 0-400 m (Fig 4). This type of fertilization contributed less to the total carbon reserves in soil, averaged for the 60 cm layer. The fertilization variants involving phosphorus and phosphorus plus potassium in the norms and ratios investigated in this study had more significant effect on the increase of these reserves; in this case there was an average increase with 18.7 % in comparison to the check variant without fertilization.

Along the soil profile, the sub-depths forming the 3rd meter had lowest Ctotal (respectively humus). No differentiation affected by the fertilization variant was found in this zone. The layers comprising the 4th meter had higher Ctotal content in comparison to the 3rd meter, and the differentiation in its content depended on the applied fertilization variant.

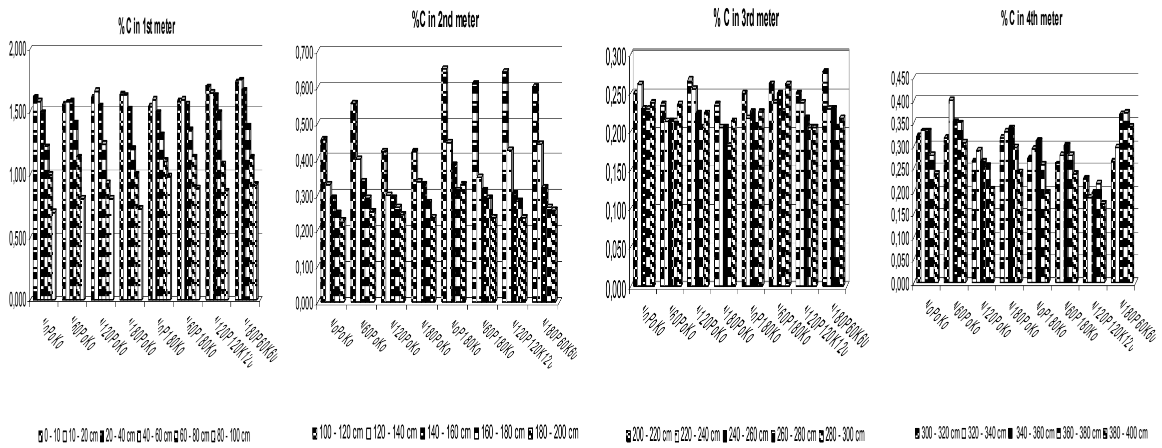

Fig. 4. Content of C_{total} (%) by layers for every meter up to 400 cm averaged for the fertilization variants

2.3.2 Soil organic mater composition along the soil profile to 400 cm depth

The variance analysis of the composition of the soil organic substance down the layers of the soil profile up to depth 400 cm revealed the dynamics in the degree of significance of the changes of C in the respective groups and fractions as a result from the long-term mineral fertilization. Although the indices of variations of the respective fertilization variants were not significant, the established differences between the investigated fertilization combinations were significant to a maximum degree, averaged for depth 0-400 cm.

Depth cm	$C_{organic}$	Humic acides (HA)	Fulvic acides (FA)	HA/FA	HA-Ca	HA-R_2O_3	Humin	FA in H_2SO_4
0-20	,000	,000	,000	,000	,000	,000	,000	,001
20-40	,000	,000	,000	,000	,000	,055	,023	,006
40-60	,000	,102	,008	,001	,052	,000	,000	,000
60-80	,000	,001	,006	,000	,001	,000	,000	,006
80-100	,000	,000	,001	,000	,000	,013	,002	,001
100-120	,001	,003	,001	,001	,003	,001	,001	,000
120-140	,002	,004	,006	,000	,003	,001	,001	,001
140-160	,001	,000	,000	,000	,000	,001	,001	,001
160-180	,000	,000	,001	,001	,000	,000	,000	,008
180-200	,002	,000	,005	,000	,000	,000	,007	,239
200-220	,003	,000	,381	,073	,000	,000	,009	,003
220-240	,001	,000	,004	,000	,000	,000	,107	,001
240-260	,000	,000	,022	,069	,000	,000	,011	,000
260-280	,008	,003	,008	,023	,001	,003	,007	,000
280-300	,002	,000	,012	,022	,000	,000	,032	,001
300-320	,004	,003	,001	,009	,007	,000	,074	,003
320-340	,000	,000	,000	,000	,000	,000	,000	,008
340-360	,000	,000	,000	,000	,000	,026	,001	,000
360-380	,000	,000	,000	,000	,000	,007	,002	,001
380-400	,000	,000	,000	,000	,000	,000	,000	,000
0-400 cm	**,000**	**,000**	**,000**	**,000**	**,000**	**,000**	**,000**	**,000**

Table 12. Variance analysis of the soil organic matter composition after a 40-year mineral fertilisation

Organic C of soil was also subjected to dynamic changes averaged for the entire 4 m depth. In this index the differentiation between the variants was less expressed in comparison to total C. Its amount was lowest in the untreated check variant.

Highest differentiation in the content of organic C according to the type of the fertilization variant was established in layers 40-60 cm and 60-80 cm, and lowest variation was found in the 260- 280 cm layer.

The independent nitrogen fertilization with 180 kg N/ha contributed most significantly to the increased amount of $C_{organic}$ averaged for a considerable depth down the profile. In this case, however, C_{humin} had lowest values. A similar tendency was found in the independent nitrogen fertilization with 120 kg N/ha, as well. In these two variants the amount of C_{humin}, also called "guard of humus", was below the level of the check variant. The independent nitrogen fertilization with 60 kg N/ha, the independent phosphorus fertilization with 180 kg P_2O_5/ha, the combination between them and the systematic balanced introduction of NPK at norm 120 kg/ha did not in practice affect the insoluble fraction of organic substance of soil under systematic agricultural cultivation of the land. Not only in the respective layers, but also in the entire 0-400 cm depth, the long-term independent nitrogen fertilization with 120 and 180 kg/ha lead to lower amounts of the insoluble residue. This is valid to a higher degree for the norm 180 kg/ha. Lowest differentiation in the values of Cresidue between the fertilization variants was determined in the 320-340 cm layer. Highest variations between the fertilization variants were established in the 0-20 cm, 60-80 cm and 380-400 cm layers.

The systematic introduction of $N_{180}P_{60}K_{60}$ had most significant contribution for $C_{residue}$ increase average for 0-400 cm profile.

Depth cm	$N_0P_0K_0$	$N_{60}P_0K_0$	$N_{120}P_0K_0$	$N_{180}P_0K_0$	$N_0P_{180}K_0$	$N_{60}P_{180}K_0$	$N_{120}P_{120}K_{120}$	$N_{180}P_{60}K_{60}$
0-20	,7969 a	,8940 c	,7868 a	,8962 c	,8284 b	,8827 c	,8949 c	,9461 d
20-40	,7824 a	,8209 b	,7625 a	,8962 d	,7782 a	,8705 c	,8583 c	,8660 c
40-60	,6539 b	,7235 d	,5919 a	,6954 c	,7406 de	,7730 f	,7486 e	,7479 e
60-80	,5172 a	,5773 cd	,5311 ab	,5448 abc	,7782 f	,5901 de	,5657 bcd	,6245 e
80-100	,3556 a	,3947 b	,3971 b	,4694 c	,5021 cd	,5047 d	,5047 d	,4818 cd
100-120	,2312 ab	,3244 d	,2498 bc	,2059 a	,3264 d	,3219 de	,2853 cd	,3026 de
120-140	,1318 a	,1632 ab	,2498 d	,2508 d	,2385 cd	,1951 bc	,2121 cd	,2227 cd
140-160	,1572 ab	,1617 abc	,2498 d	,1933 bc	,2008 c	,2438 d	,1268 a	,1424 a
160-180	,1697 b	,2348 cd	,2583 d	,2222 c	,1883 b	,2926 e	,1268 a	,1172 a
180-200	,1074 a	,2348 d	,1249 a	,2008 cd	,1757 bc	,1951 cd	,1390 ab	,0990 a
200-220	,1499 c	,1349 bc	,1413 c	,2008 d	,1255 bc	,0834 a	,1146 abc	,0990 ab
220-240	,1324 d	,1267 cd	,1357 d	,1255 cd	,0628 a	,0771 a	,0866 ab	,1053 bc
240-260	,0949 a	,0780 a	,1385 b	,2008 c	,0753 a	,0992 a	,0829 a	,0743 a
260-280	,0824 a	,1125 ab	,1520 b	,1506 b	,1004 a	,0708 a	,0829 a	,0806 a
280-300	,0999 ab	,1267 b	,1291 b	,1933 c	,1004 ab	,1244 b	,0829 a	,0619 a
300-320	,1199 ab	1389 bc	,1745 cd	,2008 d	,1255 ab	,0975 ab	,0902 a	,1231 ab
320-340	,1449 b	,2099 c	,0804 a	,3306 d	,1255 b	,1146 ab	,1073 ab	,1261 b
340-360	,1449 bc	,1876 d	,1291 abc	,3393 e	,1506 c	,1146 a	,1317 abc	,1240 ab
360-380	,1449 cd	,1632 d	,1269 bc	,2987 e	,1506 cd	,1204 ab	,1024 a	,1177 ab
380-400	,1574 c	,1369 bc	,1047 ab	,2436 d	,1506 c	,0975 a	,1354 bc	,1055 ab
0-400 cm	**,2585 a**	**,2972 c**	**,2757 b**	**,3429 d**	**,2962 c**	**,2934 c**	**,2740 b**	**,2784 b**

Table 13. Content of $C_{organic}$ by layers up to 400 cm according to the fertilization variants

Depth cm	$N_0P_0K_0$	$N_{60}P_0K_0$	$N_{120}P_0K_0$	$N_{180}P_0K_0$	$N_0P_{180}K_0$	$N_{60}P_{180}K_0$	$N_{120}P_{120}K_{120}$	$N_{180}P_{60}K_{60}$
0-20	,8055 d	,6837 a	,8779 e	,7366 bc	,7667 c	,7124 ab	,7553 c	,8085 d
20-40	,7490 bc	,7655 bc	,7892 c	,6235 a	,7154 abc	,6840 ab	,7716 bc	,8045 c
40-60	,5700 b	,6889 d	,6494 cd	,5053 a	,5762 b	,5785 b	,7479 e	,6384 c
60-80	,4805 c	,5654 e	,4028 b	,4616 c	,3326 a	,5526 de	,5132 cde	,5096 d
80-100	,3492 b	,4146 bc	,4150 bc	,2585 a	,4840 d	,3856 bc	,3653 b	,4376 cd
100-120	,2241 b	,2325 b	,1707 a	,2147 ab	,3262 cd	,2901 c	,3586 d	,2979 c
120-140	,1989 bc	,2370 c	,0489 a	,0885 a	,2082 bc	,1558 b	,2142 c	,2211 c
140-160	,1329 b	,1777 bc	,0402 a	,1344 bc	,1820 c	,0694 a	,1748 bc	,1767 bc
160-180	,0798 b	,0552 ab	,0415 ab	,0563 ab	,1250 c	,0206 a	,1545 c	,1497 c
180-200	,1188 c	,0204 a	,1216 c	,0341 ab	,1549 c	,0427 ab	,0988 bc	,1592 c
200-220	,0995 b	,1000 b	,1256 bc	,0341 a	,1239 bc	,1777 c	,1348 bc	,1795 c
220-240	,1286 abc	,0880 ab	,1196 abc	,0804 a	,1548 bc	,1608 c	,1513 abc	,1239 abc
240-260	,1342 bc	,1367 bc	,0848 ab	,0341 a	,1509 c	,1502 c	,1346 bc	,1549 c
260-280	,1467 c	,1021 bc	,0540 ab	,0321 a	,1084 bc	,1555 c	,1230 c	,1254 c
280-300	,1379 b	,1083 b	,0942 ab	,0313 a	,1258 b	,1366 b	,1230 b	,1557 b
300-320	,2020 c	,1802 bc	,0924 a	,1182 ab	,1471 abc	,1635 abc	,1389 abc	,1438 abc
320-340	,1886 b	,1904 b	,2096 b	,0460 a	,1674 b	,1667 b	,0783 a	,1726 b
340-360	,1857 b	,1663 b	,1377 b	,0624 a	,1597 b	,1870 b	,0655 a	,2473 c
360-380	,1364 bc	,1877 c	,1283 bc	,0277 a	,1075 b	,1610 bc	,1151 b	,2565 d
380-400	,0804 abc	,1706 e	,1012 bcd	,1330 cde	,0466 ab	,1403 de	,0387 a	,2483 f
0-400 cm	**0,2557 c**	**0,2629 c**	**0,2339 b**	**0,1860 a**	**0,2569 c**	**0,2544 c**	**0,2641 c**	**0,3000 d**

Table 14. Content of $C_{residue}$ by layers up to 400 cm according to the fertilization variants

The percent of $C_{organic}$ in comparison to C_{total} of soil varied within a wide range: from 43.11 % in the variant with $N_{180}P_{60}K_{60}$ to 71.51% in $N_{18}P_0K_0$. With the exception of systematic introduction of $N_{180}P_{60}K_{60}$, all other fertilization variants involved in the study contributed to the higher percent of the total humus substances in C_{total} of soil. The results from the investigation on the changes of organic C of soil showed that the independent nitrogen fertilization, especially with annually applied high norms, had a strong negative effect on the mobility of the organic substance and caused serious decrease of the percent of carbon in the insoluble residue.

The carbon of humic and fulvic acids (HA and FA) also varied considerably depending on the mineral fertilization (Fig.5). Regardless of the lower values of C of HA and FA down the soil profile, the variations between the fertilization variants were significant to a maximum degree, averaged for 0-400 cm depth. They were not significant for C-HA in the 0-40 cm layer and for C-FA in the 200-220 cm and 240-260 cm layers. Highest differentiation in the values of C-Ha between the fertilization variants was found in the 80-100 cm and 360-380 cm layers, and of the values of C-FA – in the 360-380 cm layer. Averaged for the investigated depth of 400 cm, variant $N_{180}P_0K_0$ had highest content of C-HA, exceeding the check variant with 24.9 %. A considerable increase of C-FA according to the check was determined after systematic application of $N_{180}P_0K_0$ – with 60.1 %, of $N_{60}P_{180}K_0$ – with 72.3 %, and of $N_{180}P_{60}K_{60}$ – with 70.0 %.

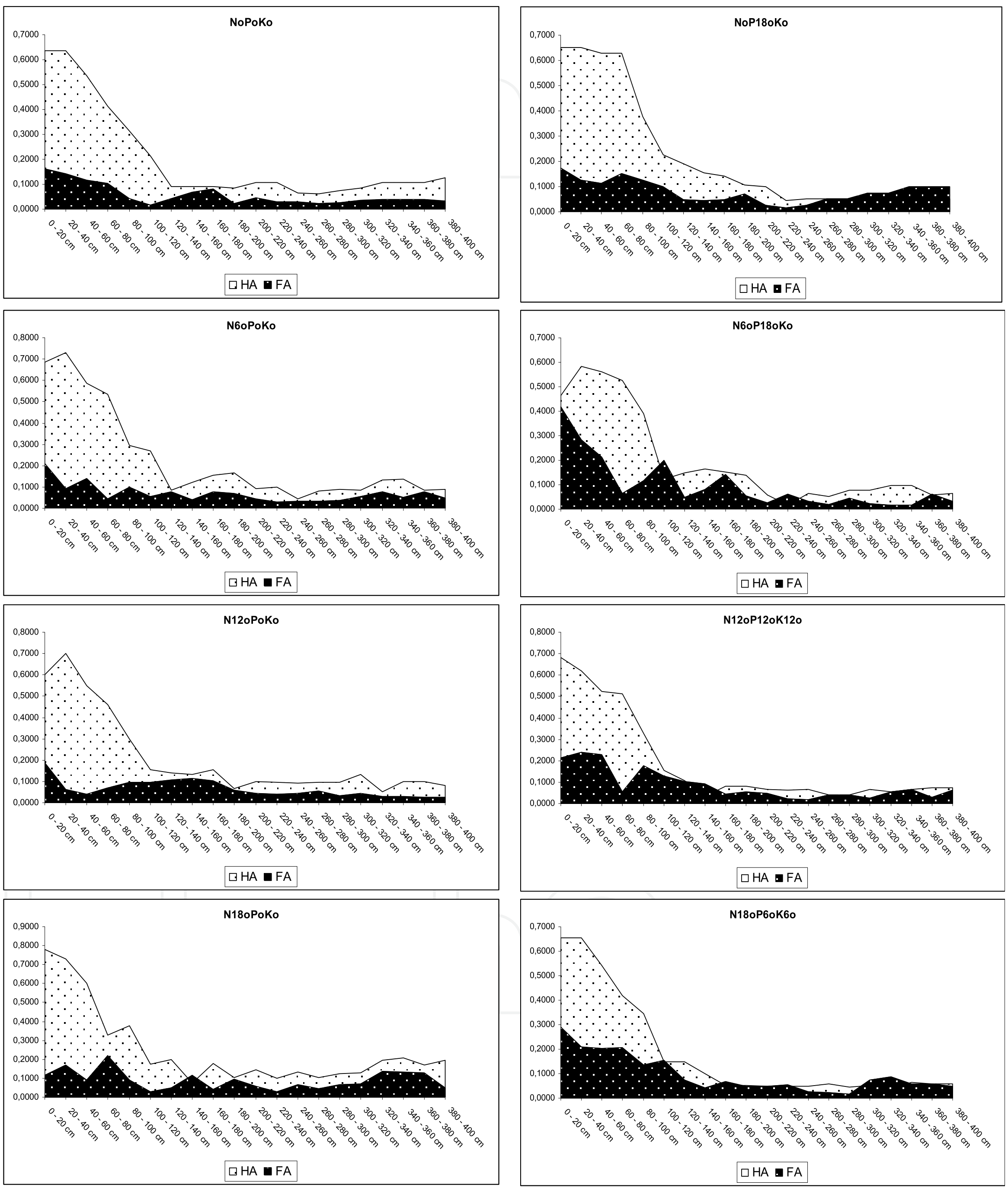

Fig. 5. Content of C_{HA} and C_{FA} by layers up to 400 cm according to the fertilization variants

The variants with independent introduction of all three nitrogen norms as well as of phosphorus had more C in HA in comparison to the check, as well as the variants with combinations of the main macro elements. The differentiation with regard to C content in FA was even better expressed. The check variant and the independent nitrogen fertilization with 60 and 120 kg N/ha had lowest amounts. In all other variants with combinations of the three macro elements, as well as in independent introduction of the highest nitrogen norm, the amount of FA increased. It reached maximum values after systematic application of $N_{60}P_{180}K_0$, similar to the fraction of aggressive FA. Averaged for this high depth profile, a tendency was observed towards higher HA content as a result from the long-term systematic mineral fertilization regardless of the norms and ratios of the main macro elements.

The changes in the content of HA and FA averaged for the 400 cm profile led to distinct differentiation between the fertilization variants with regard to the values of the ratio HA/FA (Fig.6). Variation was within a wide range: from 1.72 to 3.75. The long-term systematic nitrogen fertilization with 180 kg N/ha in combination with low fertilization norms of phosphorus and potassium determined the type of humus as fulvic-humic, averaged for the investigated depth 0-400 cm. In all other fertilization variants, regardless of the norms and ratios between the macro elements and in the check variant, the values of this ratio were above 2, which determined the type of humus as humic.

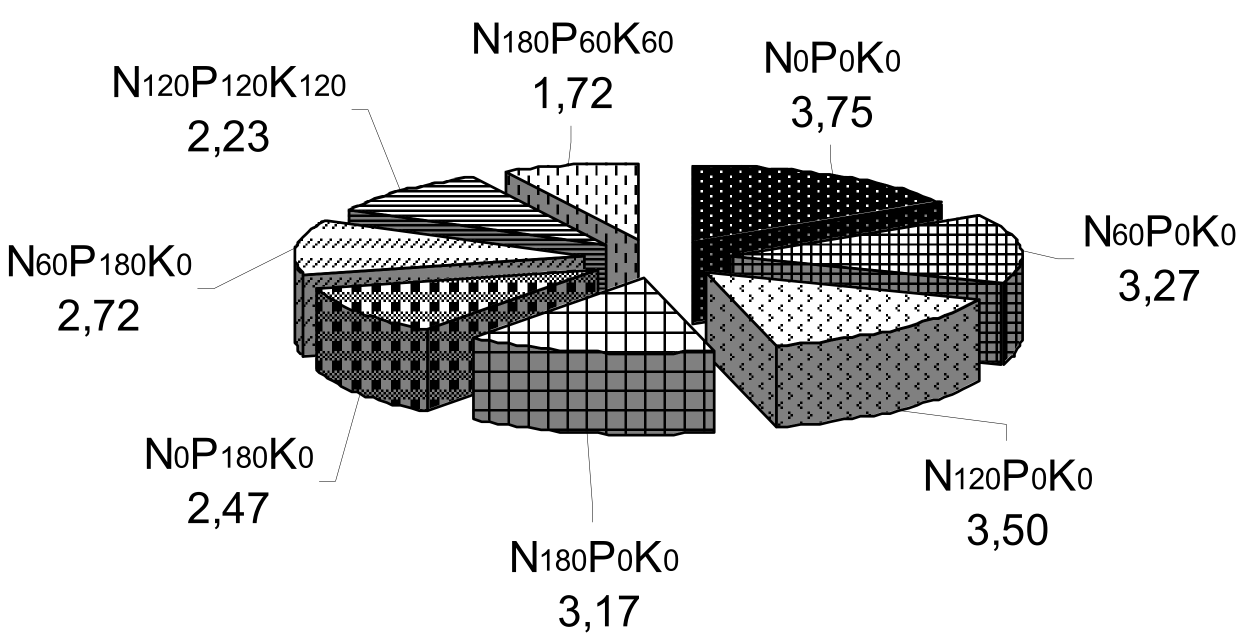

Fig. 6. Values of the ratio HA/FA average for the 0- 400 cm depth according to the fertilization variants

The similarities found between the separate fertilization variants with regard to the amount of HA linked to calcium were the reason for the lower rate of differentiation in their values. Systematic fertilization with $N_{180}P_{60}K_{60}$ and $N_{120}P_{120}K_{120}$ led to lower amount of HA-Ca, below the level of the check and all other investigated fertilization variants (Fig. 7). There was a well expressed tendency towards increase of carbon in HA-Ca as a result from the independent phosphorus and nitrogen fertilization, regardless of the nitrogen norm. Averaged for the 400 cm depth, the independent nitrogen fertilization with 180 kg/ha contributed most to the higher carbon in HA-Ca. The greater amounts of C_{HA} were due to this higher content of C in the HA fraction linked to calcium.

As a result from the long-term mineral fertilization averaged for the investigated depth, the degree of humification of the organic substance (OS) varied according to the type of the fertilization variant. The differentiation in the values of this index was extremely high regardless of the similarity and sameness in some of the variants. The 4 m profile had "very high" degree of humification of OS after systematic independent fertilization with 120 and 180 kg N/ha. Only at systematic application of NPK=3:1:1 the rate of humification averaged for the investigated profile was lowest (25.57 %) and according to Orlov and Grishina (1981) can be considered "moderate". In the other variants the values were 30-40 %, which determined humification as "high". It should be noted that in the check variant and in the variant with independent nitrogen fertilization with 60 kg/ha, the humification rate was at the upper limit tending towards "very high", while in the independent phosphorus fertilization and in the variants with $N_{60}P_{180}K_0$ and $N_{120}P_{120}K_{120}$ the values were closer to the lower limit.

Averaged for the tested variants of long-term fertilization, the slightly leached chernozem soil in the trial field can be referred to the low humic type according to the classification of Orlov and Grishina. C_{total} was highest in the 0-20 cm layer (1.62 %) and gradually decreased down the 4-m profile. In the last investigated layer (380-400 cm) its content was 0.26 %, but the 260-280 cm layer had lowest content.

Along the entire investigated profile, organic C was represented by humic acids which exceeded fulvic acids. The amount of HA was highest in the 0-20 cm layer and gradually decreased down the profile and at 380-300 cm it was 0.0919 %. Similar to organic carbon (total humic substances, THS), the amount of HA also slightly increased at depth below 300 cm. The above tendencies remained the same with regard to the changes in FA down the profile. According to the classification of Orlov and Grishina (1981), the slightly leached chernozem soil in the trial field possessed high to very high content of HA, expressed as percent from the organic C in soil. The values of this ratio were more than 80 % at depth from 20 to 80 cm. They gradually decreased down the profile but nevertheless remained within 60 – 80 %. The greater part of HA along the entire 400 cm profile was linked to calcium. Down the profile their amount gradually decreased and in the 220-300 cm zone the lowest concentrations were detected. At the 4th meter their concentration slightly increased. At depth 40-100 cm the amount of HA-Ca was very high (>80 % of HA). At all other depths the percent of HA-Ca/HA was 60-80 % and can be considered high. The amount of C in HA, free and linked to R_2O_3, was lower than the amount of the HA linked to Ca and also slightly decreased down the profile, the lowest values being registered in the 320-340 cm layer especially at $N_{180}P_{60}K_{60}$.

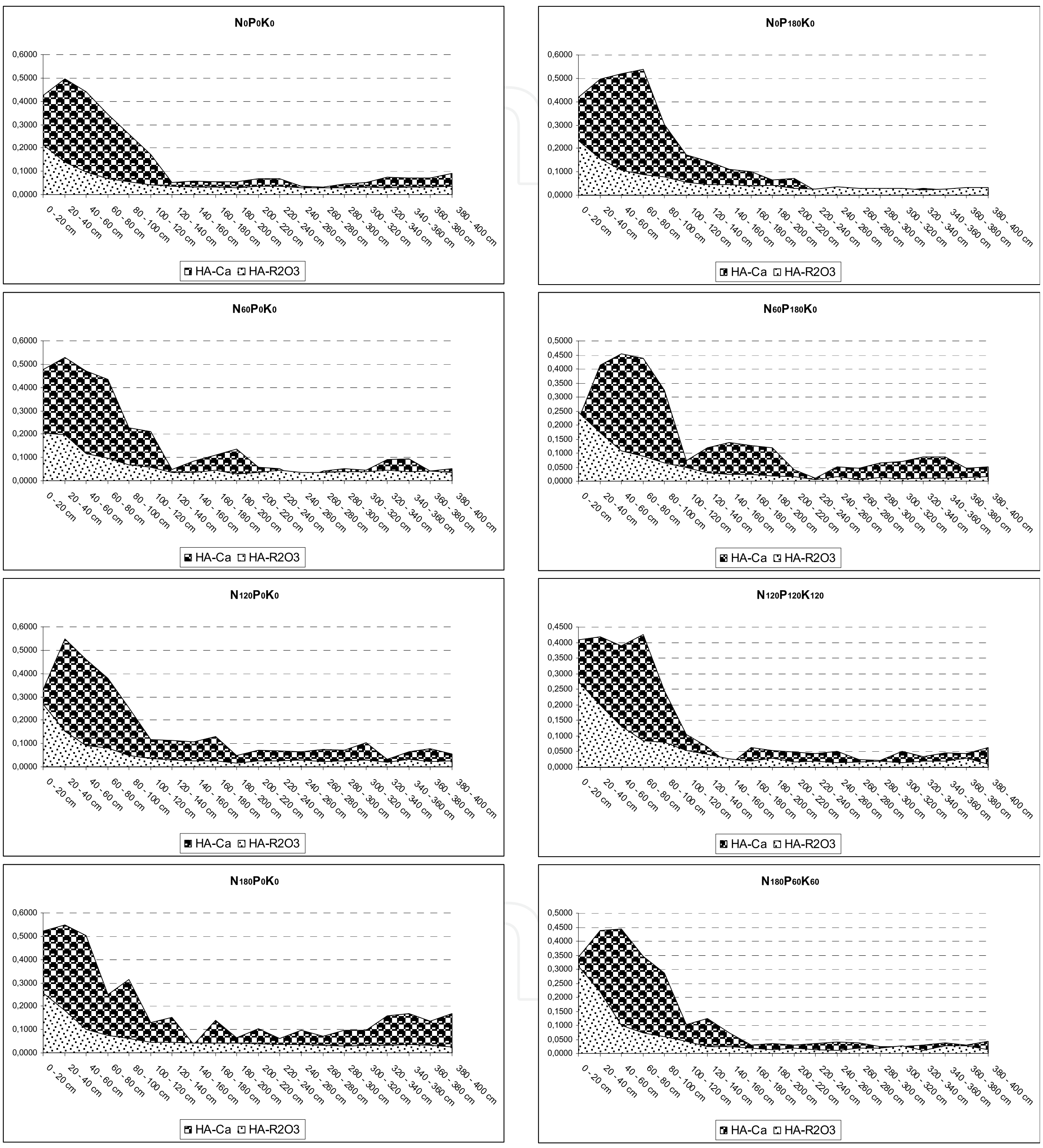

Fig. 7. The amount of C in HA, linked to Ca and free and linked to R_2O_3 by layers up to 400 cm according to the fertilization variants

2.3.3 Soil organic matter reserves in depth up to 60 cm

The systematic introduction of macro elements at different norms and ratios during a period of 40 years of cultivation of the trial field lead to formation of different reserves of total carbon in soil at depth up to 60 cm with well expressed differentiation (Fig 8). The long-term 2-field agricultural use of the trial field without mineral fertilization was characterized with lowest reserves of total C. The independent nitrogen fertilization with increasing norms caused their increase according to the check variant with 12.6 %.This increase, however, was lower than the increase registered in all other variants. Highest reserve in absolute values at the moment of taking samples was found in the variants with 40-year fertilization with $N_0P_{180}K_0$ and $N_{60}P_{180}K_0$. The main reason for this fact is that besides the variation in the content of total C, respectively humus, variation in the values of the other component was found when determining reserves – volume density of soil. According to Yankov (2007, Personal Communication), highest values of volume density averaged for the 0-60 cm layer were demonstrated by the variant with systematic introduction of phosphorus (180 kg/ha) – 1.43 g/m^3, and lowest mean values – by the variant with $N_{180}P_{60}K_{60}$ (1,22 g/m^3). Over 36 % of the total carbon reserves in soil at depth up to 60 cm were concentrated in the 20-40 cm layer, followed by the layer lying beneath (Table 12). Regardless of the low differentiation in the content of total C down the soil profile up to the 60th cm, the differentiation of the layers according to their reserves was very well expressed. Humus reserves in soil were highest in the 20-40 cm layer. The layer 10-20 cm have a negative C balance according to check variant. The maximum increase according to the control in 0-10 cm and 10-20 cm layer was established in the variants with $N_{180}P_0K_0$ and $N_0P_{180}K_0$ (136,3 and 135,6 %, respectively for 0-10 cm layer and 103,2 % and 105,3 % for 10-20 cm layer).

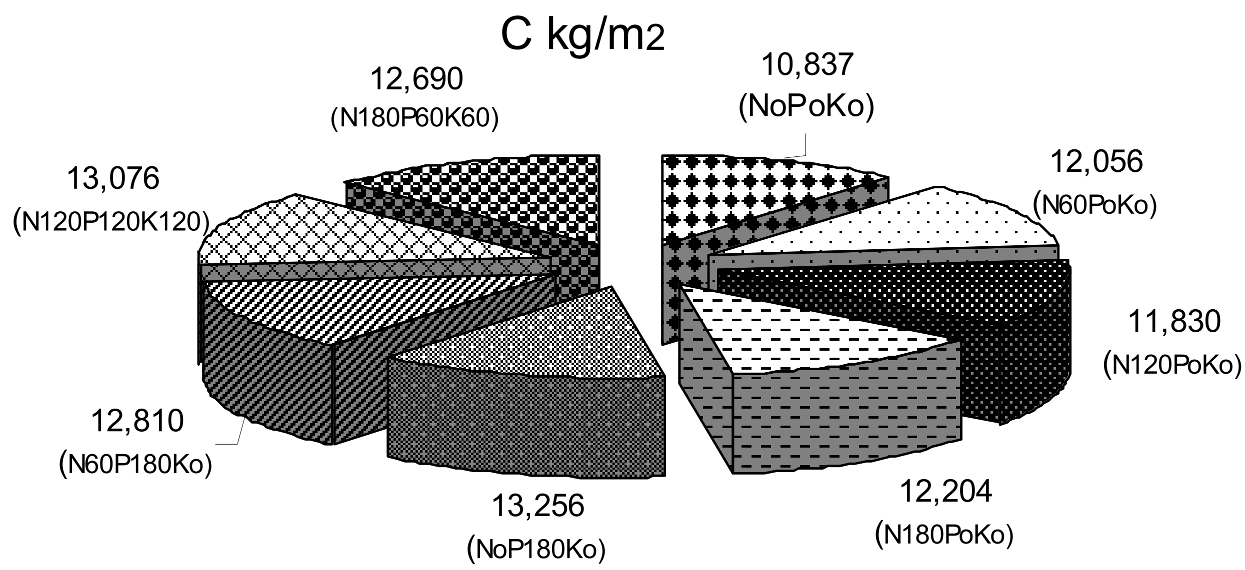

Fig. 8. Total Carbon reserves for layer 0-60 cm, C kg/m^2

Combination between macroelements affected positively the humus reserves in soil. The variant with balanced fertilization $N_{120}P_{120}K_{120}$ contributed enrichement of soil carbon reserves in 40-60 cm layer with 37,1% according to the same layer in check variant. This tendency was established also for long-term fertilization with $N_{180}P_{60}K_{60}$.

Soil depth, cm	$N_0P_0K_0$	$N_{60}P_0K_0$	$N_{120}P_0K_0$	$N_{180}P_0K_0$	$N_0P_{180}K_0$	$N_{60}P_{180}K_0$	$N_{120}P_{120}K_{120}$	$N_{180}P_{60}K_{60}$
0 – 10	1,659	1,762	1,955	2,262	2,250	2,162	2,069	1,801
10 - 20	2,323	2,104	2,301	2,397	2,446	2,080	2,127	2,240
20 - 40	3,694	4,233	4,593	4,384	4,754	4,891	4,548	4,645
40 - 60	3,161	3,957	2,981	3,161	3,807	3,677	4,333	4,005

Table 15. Reserves by depth up to 60 cm according to fertilization, C – kg/m_2

3. Conclusion

The systematic mineral fertilization for a period of 40 years with different norms and at different ratios between nitrogen, phosphorus and potassium had high effect on the agrochemical condition of slightly leached chernozem (Haplic Chernozems) down the soil profile.

The soil acidity forms, averaged for the investigated depth of the 0-400 cm profile, were significantly affected by the type of fertilizer combination. The depth was the factor with decisive effect in all forms of soil acidity. Influence of mineral fertilization was higher on exchangeable Al^{3+}, Ca^{2+} and the $\sum$Ca+Mg, and significantly lower - on the values of residual hydrolytic acidity and the rate of alkali saturation. The amount of exchangeable Mg^{2+} had a clear tendency toward increasing down the soil profile

Independent long-term mineral fertilization with 180 kg N/ha and with $N_{180}P_{60}K_{60}$ caused the occurrence of exchangeable Al^{3+} in the soil absorption complex in the surface layers 0-10 and 10-20 cm. It was not present further down the profile.

The variant with independent nitrogen fertilization with 180 kg/ha has also the lowest $\sum$Ca+Mg and the lowest value of sorption capacity. Intensive mineral fertilization with $N_{180}P_{60}K_{60}$ caused decreasing of degree of saturation with bases.

The value of the pH, sorption capacity, acidity on strongly acid positions and degree of saturation with bases showed a tendency of decreasing at the end of 40th year of trail beginning comparing the end of 30th year. In the same way independently of fertilizer variant the acidity on the slightly acid positions is strongly increased.

The mineralization ability down the soil profile was affected to a maximum degree of significance by the mineral fertilization and the incubation. Depth had decisive effect on the value of the index. The maximum effect of this factor was registered after 28-day incubation – 92.3 %. The role of mineral fertilization on nitrogen mineralization according to the incubation period was significantly less expressed – 9.2 %, 3.2 5 and 4.0 %, respectively. The amount of nitrified nitrogen increased with the longer incubation periods with 52.7 % (28 days) and with 118.8 % (56 days), respectively, in comparison to 14-day incubation. The long-term mineral fertilization with $N_{180}P_{60}K_{60}$ had the highest values of mineralization ability for all three incubation periods. The established strong effect of systematic mineral fertilization regardless of the norm and ratios of nitrogen, phosphorus and potassium on the mineralization ability of soil in comparison to the check variant was highest at depths 0-100 cm and 300 – 400 cm.

Systematic mineral fertilization carried out for 40 years in two-field crop rotation (wheat – maize) affected the content of C_{total} deep down the profile of the slightly leached chernozem soil. Systematic use of $N_{180}P_{60}K_{60}$ contributed most for the increase of its content at average depth 0-400 cm. Fertilizer variants $N_{180}P_{60}K_{60}$ and $N_{120}P_{120}K_{120}$ led to lower amount of HA-Ca, below the level of the check and all other investigated fertilization variants. There was a well expressed tendency towards increase of carbon in HA-Ca as a result from the independent phosphorus and nitrogen fertilization, regardless of the nitrogen norm.

The ratio C_{HA}/C_{FA} putted under average for the 400 cm profile to distinct differentiation between the fertilization variants.Variation was within a wide range: from 1.72 to 3.75. The long-term systematic nitrogen fertilization with 180 kg N/ha in combination with low fertilization norms of phosphorus and potassium determined the type of humus as fulvic-humic, averaged for the investigated depth 0-400 cm. In all other fertilization variants, regardless of the norms and ratios between the macro elements and in the check variant, the values of this ratio were above 2, which determined the type of humus as humic. Independent nitrogen fertilization, especially with annually applied high norms, had a strong negative effect on the mobility of the organic substance and caused serious decrease of the percent of carbon in the insoluble residue.

As a result from the systematic mineral fertilization in the 20-40 cm layer, higher reserves were formed by the layers lying above and below. Triple NPK combinations ($N_{120}P_{120}K_{120}$ and $N_{180}P_{60}K_{60}$) enriched organic mater reserves in 40-60 cm layer.

These results showed that regardless of the intensive agricultural activities and changes of some agrochemical characteristics, slightly leached chernozem soil (Haplic chernozems) in Sough Dobrudzha region in Bulgaria preserved its main genetic characteristics at the lower depths of the soil profile.

The effect of long-term fertilizer treatments on detail nutrient balances, technological quality of crops, concentration of available forms of macro elements and trace elements in soil and plant biomass dynamics and many other aspects of this experiment await detailed analysis.

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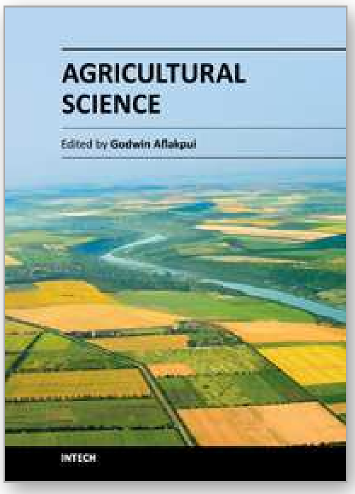

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This book covers key areas in agricultural science, namely crop improvement, production, response to water, nutrients, and temperature, crop protection, agriculture and human health, and animal nutrition. The contributions by the authors include manipulation of the variables and genetic resources of inheritance of quantitative genes, crop rotation, soil water and nitrogen, and effect of temperature on flowering. The rest are protecting crops against insect pests and diseases, linking agriculture landscape to recreation by humans, and small ruminant nutrition. This book is a valuable addition to the existing knowledge and is especially intended for university students and all professionals in the field of agriculture.