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Changes in the total nitrogen content down the profile of slightly leached chernozem soils as a result from 40-year mineral fertilization

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Abstract
The systematic mineral fertilization for a period of 40 years with various nitrogen, phosphorus and potassium norms and ratios had high effect on the agro-chemical status down the profile of the slightly leached chernozem soil (0 – 400 cm). Systematic mineral fertilization applied for 40 years affected significantly the content of total nitrogen in soil (averaged for the 0 - 400 cm layer). There was a clear negative effect of independent fertilization with $N_{180}P_0K_0$ on total nitrogen, which is one of the stable indicators of soil fertility. The mean decrease according to the control variant was with 4.8%. Highest enrichment of soil with total nitrogen along the entire investigated depth was found after fertilization with $N_{60}P_{180}K_0$ (4.1%) and $N_{120}P_{120}K_{120}$ (6.6%).

The differentiation in the values of total nitrogen along the entire profile was well expressed, the changes in the 1st meter being most dynamic. Lowest total nitrogen content was detected in the 3rd meter.

Key words: Total nitrogen concentration, long-term mineral fertilization, soil profile up to 400 cm

Introduction
Transportation, redistribution and transformation of nitrogen down the soil profile were influenced 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 aim of this investigation was to follow the effect of the long-term agronomy practices and especially fertilization on the total nitrogen concentration of slightly leached chernozem soil in the region of South Dobrudzha after 40 years of mineral fertilization with different norms and combination between nitrogen, phosphorus and potassium.

Materials and methods
Dobrudzha Agricultural Institute-General Toshevo is situated in the north-eastern part of Bulgaria in the zone of black earth (Picture 1). The main soil type is chernozem (Haplic Chernozems WRBSS, 2006). A long-term fertilizer experiment, which was initiated in 1967, is still carried on. In a two-field crop rotation (wheat-maize), 4 nitrogen and phosphorus and 3 potassium norms were tested – 0, 60, 120, 180 and 0, 60, 120 kg/ha respectively.

Picture 1. Location of Dobrudzha Agriculture Institute (43^{o} 40’ northern latitude and 28^{o} 10’ eastern longitude)

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 and Nankova, 1994; Iliev, 2000). The changes of total nitrogen concentration were determined in selected variants which possessed highest productivity average for the 40thyear period of investigation.

Total nitrogen content was determined by the classical method of Kjeldahl. The mathematical analysis of the obtained results was performed with the help of Excel and the software SPSS 16.0 (2007). The post-hoc analyses were expressed through the Waller-Duncan test ($P<0.05$).

Results and discussions

The investigations on the ecological status of the slightly leached chernozem in South Dobrudzha included mostly the systematic and long-term effect of some agronomy practices, predominantly of mineral fertilization (Nankova et al., 1994, 2005; Nankova, 2010). For the first time in this region of Bulgaria an in-depth investigation was carried out on the influence of the long-term mineral fertilization on the distribution of total nitrogen by layers every 20 cm till depth 4 m.

The total nitrogen concentration was significantly affected by the mineral fertilization and the investigated layers up to depth 400 cm (Table 1). At the end of the studied period, averaged for the depth of the 0-400 cm profile, a significant effect was established to a maximum degree both under the independent influence of the investigated factors and under their interaction.

The depth of the investigated profile was the factor with decisive effect on soil nitrogen concentration (Figure 1).

Table 1. Variance analysis of the total nitrogen concentration for a 40-year period of investigation

Source	df	Mean Square	F	Sig.
Fertilizer variants (A)	7	225.764	19.563	0.000
Soil depth (B)	20	37302.907	3232.358	0.000
A x B	140	102.156	8.852	0.000

a R Squared = 0.997 (Adjusted R Squared = 0.995)

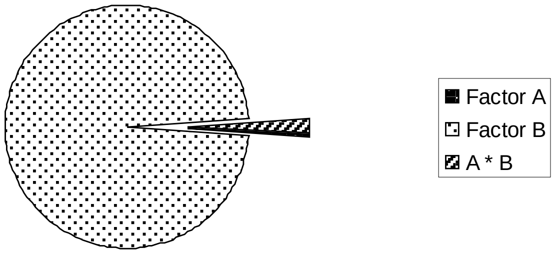

Figure 1. Power of factors influence on the total N concentration

Total N of soil was subjected to dynamic changes averaged for the entire 4 m depth. In this index the differentiation between the variants was very well expressed, similarly to total C (Nankova, 2011; Nankova et al., 2011). Its amount was lowest in the variant with independent nitrogen fertilization with 180 kg N/ha (Table 2). The established nitrogen content differences in the 0-400 cm layer in the check variant, in the variants with independent N fertilization with moderate and optimal N norms and in the variant $N_{180}P_{60}K_{60}$ were significantly smaller. Therefore they were separated into a group with well expressed similarity, with higher total N content than the variant with 40 years of systematic independent nitrogen fertilization. The variant with systematic P fertilization with 180 kg P_2O_5/ha was characterized with higher total N content averaged for the entire profile than the variants with independent N fertilization, but the results showed presence of similarity with them.

Table 2. Soil nitrogen concentration according to the fertilizer variants averaged for 0-400 cm soil profile, mg/100 g soil (Waller-Duncan N=42)

Fertilizer variants	Value	Groups
$N_0P_0K_0$	67.3	bc
$N_{60}P_0K_0$	68.3	cd
$N_{120}P_0K_0$	67.8	bcd
$N_{180}P_0K_0$	64.1	a
$N_0P_{180}K_0$	69.0	de
$N_{60}P_{180}K_0$	70.1	e
$N_{120}P_{120}K_{120}$	71.7	f
$N_{180}P_{60}K_{60}$	66.4	b

The systematic introduction of $N_{120}P_{120}K_{120}$ had most significant contribution for soil nitrogen enrichment average for the 0-400 cm layer. A similar tendency was established for dressing with $N_{60}P_{180}K_0$ but this variant formed a lower level group than the systematic balanced introduction of NPK at norm 120 kg/ha. Most probably very good conditions for microbiological activity had been created in these variants, i.e. almost neutral soil reaction, better soil structure ensuring more efficient aeration, water and nutrients supply, which contributed for improving of the soil nitrogen regime.

The distribution of the total N content after long-term mineral fertilization averaged for the fertilizer variants by the layers and meters down the soil profile showed interesting results (Table 3). The soil layers of the 1^{st} meter had highest potential nitrogen-supplying capacity. The differentiation between the layers forming this meter was very well expressed.

Table 3. Soil nitrogen content by layers up to 400 cm depth average for the fertilizer variants (mg/100 g soil) Waller-Duncan N=16

1st meter		2nd meter		3rd meter		4rd meter	
Depth cm	Value/ group	Depth cm	Value/ group	Depth cm	Value/ group	Depth cm	Value/ group
0-20	167.5 k	100-120	65.8 f	200-220	38.4 bc	300-320	38.8 bc
20-40	156.4 j	120-140	49.5 e	220-240	37.6 bc	320-340	39.3 c
40-60	131.6 i	140-160	45.3 d	240-260	33.6 a	340-360	37.2 b
60-80	109.9 h	160-180	39.5 c	260-280	32.9 a	360-380	38.2 bc
80-100	94.0 g	180-200	37.6 bc	280-300	34.6 a	380-400	34.7 a

Total N content in the layer 0-20 cm was highest and gradually decreased from 167.5 mg N/100 g soil to 94.0 mg N/100 g soil in the 80-100 cm layer. Regardless of the of the fertilizer variants, the average total N content in the 1st meter was 131.85 mg N/100 g soil. Highest total N content was established at fertilization with $N_{120}P_{120}K_{120}$ not only in the surface layer but down the soil layers in the 1st meter – an average of 142.65 mg N/100 g soil (Figure 2).

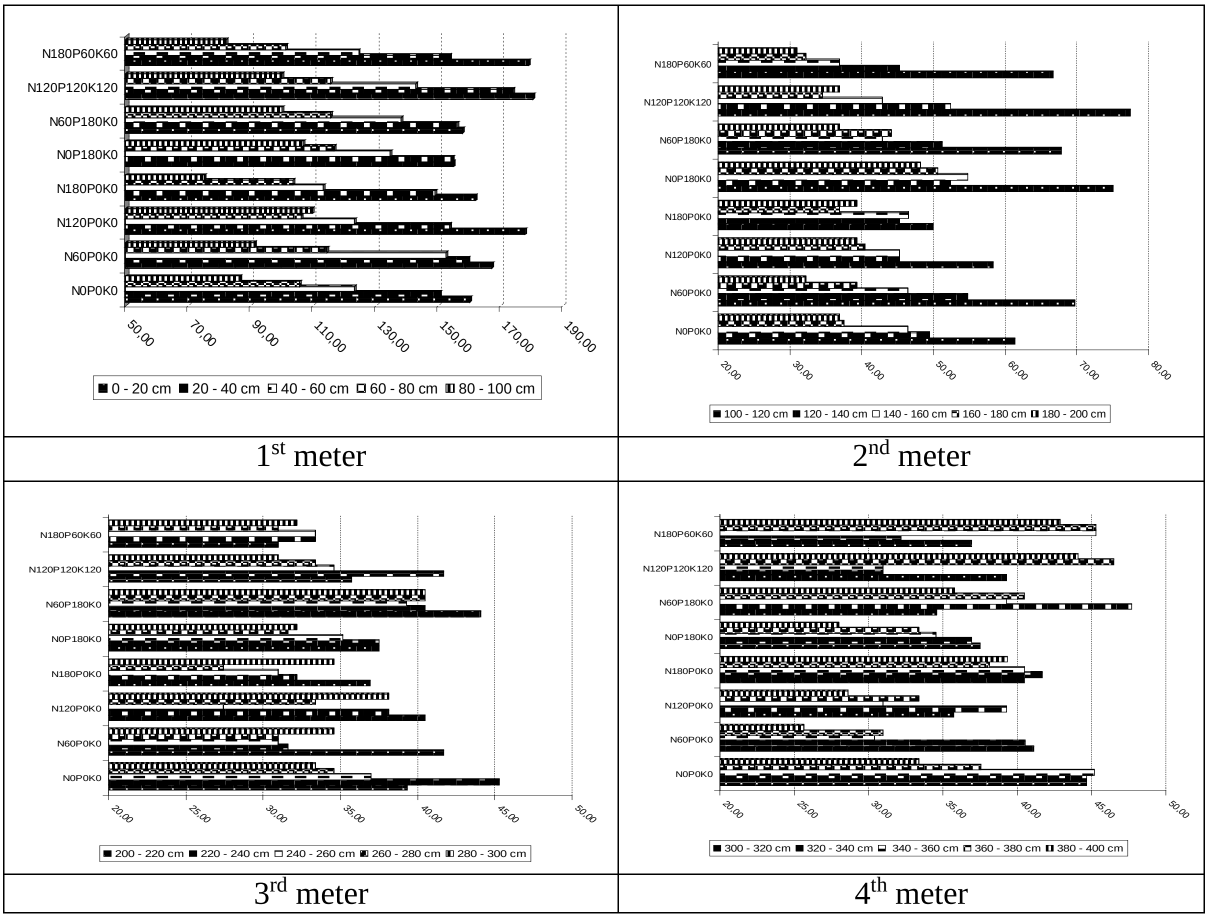

Figure 2. Total N content according to the fertilizer variants by meters, mg N/100 g soil

Annual fertilization only with 180 kg N/ha lead to decreasing of the total N content averaged for the 1st meter layers (120.61 mg N/100 g soil). The results were lower than that of the control variant. Highest mean concentration of N_{total} in the first meter was determined after systematic fertilization with $N_{120}P_{120}K_{120}$ (142.65 mg N/100 g soil). The main reason for this fact is the higher concentration of nitrogen in the layers up to 60 cm depth.

In the layers forming the 2nd meter (beginning of loess horizon) total N content decreased still further. Its average content was 47.55 mg N/100 g soil and varied from 65.84 (100-120 cm) to 37.62 (180-200 cm). The $N_{180}P_0K_0$ variant had lowest content of total N among all tested variants. The differentiation between the fertilizer variants remained the same, although at a lower level. The mean values of the tested fertilizer variants for the second meter varied from 42.43 mg N/100 g soil ($N_{180}P_{60}K_{60}$) to 56.25 mg N/100 g soil ($N_0P_{180}K_0$). The systematic independent nitrogen fertilization with moderate and high norms, as well as the combination $N_{180}P_{60}K_{60}$ lead to decrease of the mean content of total nitrogen in the second meter in comparison to the check variant. The mean value of total nitrogen content in the second meter was 33.8% from the value determined for the first meter.

Along the soil profile, the sub-depths forming the 3rd meter had lowest total N content among the tested meters. Regardless of low average N content (35.39 mgN/100 g soil) in this meter differentiation between fertilizer variants is still expressed. Long-term fertilization with $N_{180}P_0K_0$ and $N_{180}P_{60}K_{60}$ caused strongly decreasing of N content comparing with other variants.

The variants with the participation of the highest nitrogen norm had lower mean content of total nitrogen in the third meter. From all fertilizer variants, only at systematic fertilization with $N_{60}P_{180}K_0$ the mean concentration of total nitrogen in soil exceeded the values determined for the check variant. The mean content of total nitrogen in the third meter was 26.8% from the value established in the first meter, and 74.4% from the value of the second meter.

The layers comprising the 4th meter had higher total N content in comparison to the 3rd meter, and the differentiation in its content depended on the applied fertilization variant. Its average content was 37.63 mg N/100 g soil and varied from 39.26 (320-340 cm) to 34.72 (380-400 cm) average for the fertilizer variants. The differentiation affected by the fertilization variant and layers was found in this zone.

At this depth the check variant had highest mean content of total nitrogen. The variants with systematic introduction of the highest nitrogen norm were closest to it. The combined mineral fertilization with NP and NPK lead to higher concentrations of total nitrogen in the layers 360-380 cm and 380-400 cm in comparison to the check variant. Some increase of 6.3% was observed in the concentration of total nitrogen in the fourth meter according to the value established in the third meter. The mean content of total nitrogen in the fourth meter was 28.5% from the content in the first meter.

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 nitrogen in soil at depth up to 60 cm with well expressed differentiation (Figure 3). The long-term two-field agricultural use of the trial field without mineral fertilization was characterized with lowest reserves of total N. The independent nitrogen fertilization with increasing norms caused their increase according to the control variant with 14.3%, 10.6% and 6.8% respectively. Highest reserve in absolute values at the moment of taking samples was found in the variants with 40-year fertilization with $N_{120}P_{120}K_{120}$ and $N_0P_{180}K_0$. 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).

There was a clear negative effect of independent fertilization with $N_{180}P_0K_0$ on total nitrogen, which is one of the stable indicators of soil fertility. The mean decrease according to the control variant was with 4.8%. Highest enrichment of soil with total nitrogen along the entire investigated depth was found after fertilization with $N_{60}P_{180}K_0$ (4.1%) and $N_{120}P_{120}K_{120}$ (6.6%).

The differentiation in the values of total nitrogen along the entire profile was well expressed, the changes in the 1st meter being most dynamic. Lowest total nitrogen content was detected in the 3rd meter.

All fertilizer variants at depth up to 60 cm had positive N balance according to the control variant. 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. Long-term independent nitrogen fertilization with medium and high norms caused negative N balance in 40-60 cm layer.

The triple NPK balanced combination ($N_{120}P_{120}K_{120}$) enriched the nitrogen reserves to a maximum degree according to the other variants (with 22.4%).

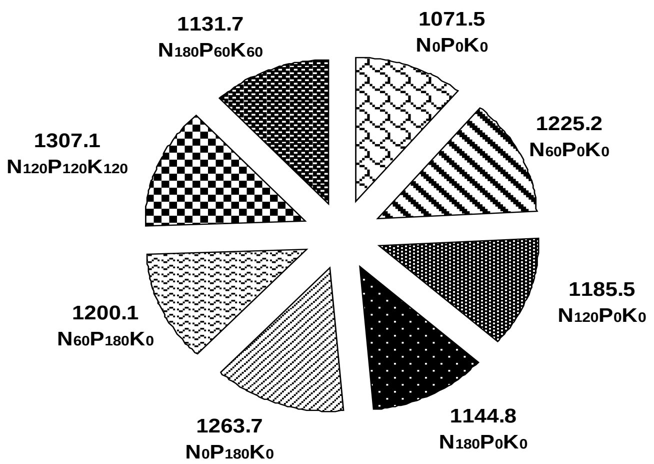

Figure 3. Total Nitrogen reserves in layer 0-60 cm, N g/m^2

Over 36% of the total nitrogen reserves in soil at depth up to 60 cm were concentrated in the 20-40 cm layer, followed by the layer lying beneath (Table 4). Regardless of the low differentiation in the content of total N down the soil profile up to the 60th cm, the differentiation of the layers according to their reserves was very well expressed. Nitrogen reserves in soil averaged for the tested variants were highest in the 20-40 cm layer. All fertilizer variants at depth up to 60 cm had positive N balance according to the control variant. The maximum increase according to the check variant in the 0-20 cm layer was established in the variants with $N_{120}P_0K_0$, $N_{180}P_0K_0$ and $N_0P_{180}K_0$ (about 13%). Nitrogen reserves in all tested fertilizer variants in the 20-40 cm layer exceeded the check variant with 110.9% ($N_{180}P_{60}K_{60}$) to 132.4% ($N_{120}P_{120}K_{120}$). The long-term independent nitrogen fertilization with moderate and high norms caused negative N balance in the 40-60 cm layer. Averaged for the investigated fertilizer variants, the increase of the established reserves of total nitrogen at depth up to 60 cm exceeded the reserves in the check variant with 12.8%.

Table 4. Increase of N reserves by depth up to 60 cm according to the check, %

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 – 20	100.0	101.2	113.4	113.4	113.3	102.4	110.7	100.0
20 - 40	100.0	115.5	122.0	114.4	126.2	126.7	132.4	110.9
40 - 60	100.0	129.7	93.8	89.6	114.1	107.0	124.1	106.6

The correlations between the content of total nitrogen in the soil averaged for the tested variants of the trail at depth up to 400 m with the content of total carbon and the group and fraction composition of the organic substance in soil were strongly expressed (Table 5). Highest values of the investigated correlations of total nitrogen were determined with total carbon, with the total humus substances and with carbon in the insoluble residue. The correlation of total nitrogen and carbon with the humin acids was better expressed than the correlation with the fulvic acids.

Most of the correlations with the respective ratios in the fraction composition of the organic substance were also highly significant. Insignificant were only the ratios THS/TC and Humin/TC.

Table 5. Correlations of total nitrogen content with the organic substance of soil at 0-400 cm averaged for the tested variants

With percent of carbon by groups and fractions									
Total C (TC)	C in THS	C_{HA}	C_{FA}	C_{HA}/C_{FA}	C_{HA} with R_2O_3	C_{HA} with Ca	C_{Humin}	C_{AFA}	
0.988 (**)	0.969 (**)	0.960 (**)	0.716 (**)	0.437 (**)	0.916 (**)	0.918 (**)	0.965 (**)	0.665 (**)	
With the respective ratios									
AFA/TC	THS/TC	HA/THS	FA /THS	HA_{R2O3}/HA	HA_{Ca}/HA	Humin/TC	HA/TC	$C_{org}/C_{residue}$	C/N
-0.581 (**)	-0.006^{NS}	0.409 (**)	-0.409 (**)	-0.195 (**)	0.195 (**)	0.006^{NS}	0.256 (**)	-0.136 (*)	0.581 (**)

THS – Total humic substances; HA – Humic acids; FA- Fulvic acids; AFA- Aggressive fulvic acids

Conclusions

The systematic mineral fertilization applied for 40 years with different norms and at different ratios between nitrogen, phosphorus and potassium affected significantly the content of total nitrogen of slightly leached chernozem (Haplic Chernozems) down the soil profile.

The content of total nitrogen in soil decreased down the investigated profile (0-400 cm) with 167.44 mg N/100 g soil averaged for the layer 0-20 cm to 32.86 mg N/100 g soil averaged for the layer 260-280 cm. Most dynamic changes in the concentration of total nitrogen of soil occurred in the first meter. Its mean content was highest at systematic balanced introduction of nitrogen, phosphorus and potassium at norm 120 kg/ha both in the first meter and for the entire investigated depth. The increase according to the check variant was with 13.9% and 5.8%, respectively.

The use of the aggressive nitrogen norm of 180 kg/ha, independently and in combination with phosphorus and potassium (N:P:K=3:1:1) caused decrease of the total nitrogen of soil at high depth down the profile.

The formed reserves of total nitrogen by layers to depth of 60 cm varied significantly. The mineral fertilization, regardless of the type, norm and ratio of nitrogen, phosphorus and potassium ensured a positive balance of nitrogen in soil according to the check variant, averaged for the investigated depth. The reserve of total nitrogen was highest at systematic fertilization with $N_{120}P_{120}K_{120}$. Along the vertical axis, the greatest part of the reserve was concentrated in the 20-40 cm layer (36.3% from the total). The 40-60 cm layer had lowest mean reserve (28.2% from the total) and its values strongly decreased at systematic nitrogen fertilization with norms 120 and 180 kg/ha.

The correlations of total nitrogen content in soil with the amount of total carbon and with the composition of the soil organic substance had high statistical significance.

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