

The changes of soil nitrogen and carbon contents in a long-term field experiment under different systems of nitrogen fertilization

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ABSTRACT

Content of N and C in soil were investigated in a long-term field experiment under different systems of N fertilization. Chernozem and Cambisol were extracted using hot water (N_{hws} , C_{hws}) and 0.01M CaCl_2 (N_{CaCl_2} , C_{DOC}). The C_t/N_t ratio in Chernozem was 9.6:1 and in Cambisol 6.1:1. The lowest C_t/N_t ratio in both experiments was found in the control treatment. Results showed that C and N compounds are less stable in Cambisol, which leads to a higher rate of mineralization. In the Chernozem, N_{hws} formed 3.66% from the total N content in the soil whereas N_{CaCl_2} formed only 0.82%. C_{hws} formed 2.98% and C_{DOC} 0.34% from total C content. Cambisol contains 4.81% of N_{hws} and 0.84% of N_{CaCl_2} from the total N amount and 5.76% of C_{hws} and 0.70% of C_{DOC} from the total C content, respectively. Nitrogen extracted by 0.01M CaCl_2 formed only 22.4% of N extractable by hot water in Chernozem and 17.5% in Cambisol. The lowest C/N ratios were obtained after the CaCl_2 extraction (3.0–6.2:1). The application of manure increased the content of soil organic N and C compared to the sewage sludge treatments.

Keyword: long-term field experiment; nitrogen; carbon; sewage sludge; farmyard manure; soil organic matter

Soil organic matter (SOM) significantly affects soil fertility (Kubát and Lipavský 2006); it consists of primary organic matter (POM) and humus. Primary organic matter has a very low cation exchange capacity, transforms during the mineralization process (POM content decreases as a result of CO_2 release) and humification and it is a source of energy for soil microbial biomass (Kolář et al. 2000). According to Körschens (1980), SOM can be divided into two fractions. The first fraction is relatively inert (hardly involved in SOM mineralization processes) and strongly correlates with contents of clay and fine particles in soils. The second one is potentially mineralizable and may be expressed as active organic carbon content (hot water soluble carbon – C_{hws}).

Ghani et al. (2003) recommended C_{hws} as an integrated indicator of soil organic matter quality. Hot water extractable carbon correlated well with contents of easily decomposable saccharides,

mineralizable nitrogen, carbon and soil microbial biomass (Körschens et al. 1990). Water used as a solvent for this extraction corresponds to natural conditions (Körschens et al. 1990, Schulz 1990) and gives real results. However C_{hws} is not a clearly described fraction of the SOM. This fraction contains parts of soil microbial biomass, simple organic compounds and compounds hydrolyzable or depolymerizable under the conditions by hot water extraction (Schulz 1997).

Annual N amount mineralized from soil organic matter can account for more than 100% (in similar conditions under annual course of weather conditions). For this reason, it is important to know the amount of N that can be mineralized from SOM over the year and to determine the optimal dose of N fertilization according to these data. Extractable nitrogen (N_{hws}) analyses using hot water in more than 1000 soils sampling from different long-term field experiments demonstrate

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Table 1. Experiment design

Number of treatment	Fertilization	Potatoes	Wheat	Barley
		(kg N/ha)		
1	control	0	0	0
2	SS1	330	0	0
3	SS3	990	0	0
4	FYM	330	0	0
5*	N	120	140 (70 + 70)	70
6*	N + straw	120 + 5 t straw	140 (70 + 70)	70

SS – sewage sludge; FYM – farmyard manure

*N in nitrochalk, 27% N

that annual N_{hws} fluctuation is higher compared to C_{hws} fluctuation. The reason of this difference is the mineral form of N (NH_4^+ , NO_3^-) – part of hot water extractable fraction, which is fluctuated during the year (Schulz 1997).

Körschens (1980) (hot water extraction), Houba et al. (1986) (0.01M $CaCl_2$ extraction) and Nemeth (1976) (EUF method) used the results of mineral N contents and the results of extracted N binding to organic substances (N_{org}). During one growing period, 60–120 kg N/ha is released by mineralization of SOM. The results from long-term field experiments confirmed that N compounds of SOM are decomposed and mineralized more easily compared to the compounds not containing N (Kubát et al. 1999). The amount of mineralizable N in soils depends on two factors. The first one is the content of mineralizable N compounds (quantity of N-pool), which is in close relation to the humus pool. The humus pool is divided into 3 groups by Paul and Juma (1981): active pool (undecomposed extinct microbial biomass and plant residues, half-life 77 weeks), stable pool (half-life 30 years) and passive pool (half-life 600 years). The second factor is then the activity of microbial processes (intensity of transformation processes). The soil microbial biomass is related to the SOM content in arable soils (Černý et al. 2008). Microbial bio-

mass is the living part of SOM and can be a good index for determination of soil changes (Houat and Chaussod 1995). New objective methods for determination of microbial biomass are developed for its significant role in soil organic matter transformation (Parkinson and Paul 1982).

The aim of this study was to compare the hot water extraction method and the 0.01M $CaCl_2$ extraction, and to evaluate the changes in contents of different N and C forms in long-term experiments under different systems of N fertilization, especially focused on fertilization with sewage sludge

MATERIAL AND METHODS

Stationary long-term field experiments were established in 1997 at 2 experimental sites with different soil-climatic conditions: in Suchdol (Chernozem) and Lukavec (Cambisol) (Table 1). There was a rotation of three crops in the following order: potatoes, winter wheat and spring barley. All three crops were cultivated each year simultaneously. Experiments consisted of 6 treatments: (1) control treatment, (2) sewage sludge 1 (SS1), (3) sewage sludge 3 (SS3), (4) farmyard manure (FYM), (5) mineral N fertilizer, (6) mineral N fertilizer + straw. Soil-climatic characteristics of both regions are showed in Table 2. Values of pH/ $CaCl_2$, content of total carbon C_t and total nitrogen N_t and content of available nutrients estimated according to Mehlich III (P, Mg, K) are showed in Table 3.

Organic fertilizers (sewage sludge, farmyard manure, straw) were applied only to the first crop (potato) in rotation. Direct effects of applied organic fertilizers were monitored for potatoes, subsequent effects for winter wheat and spring barley. During one crop rotation, 330 kg N/ha was applied for each treatment, only the control treatment was not fertilized and SS3 was applied at the rate of 990 kg N/ha. Barley straw was applied at the rate of 5 t/ha under the potatoes for the treatment 6. Mineral N-fertilizers for winter wheat were applied in two spring rates (first application

Table 2. Soil-climate conditions in experimental sites

Region	Altitude (m above sea level)	Aggregate average		Soil type	Soil class
		precipitation (mm)	temperature (°C)		
Suchdol	286	495	9.1	Chernozem	loam
Lukavec	610	666	7.7	Cambisol	sandy-loam

Table 3. pH value, contents of C_t , N_t and contents of available nutrients in year 2005 in control treatment (Mehlich III)

Soil	pH/CaCl ₂	C_t (%)	N_t (%)	Mehlich III		
				P (mg/kg)	Mg (mg/kg)	K (mg/kg)
Chernozem	7.4	2.46	0.26	78	203	120
Cambisol	5.3	1.43	0.24	152	87	219

– 70 kg N/ha, second application – 70 kg N/ha). Nitrogen for barley was applied before seeding (70 kg N/ha). Sewage sludge from the wastewater treatment plant in Prague-Troja was used for this experiment.

Hot water extraction

Extraction using hot water (Körschens et al. 1990) was used for assessment of extractable forms of soil nitrogen and extractable soil organic carbon. Soil samples were dried at 40°C and extracted with water (1:5, w/v). Suspension was boiled for one hour. The $N-NO_3^-$, $N-NH_4^+$ content, content of total extractable nitrogen (N_{hws}) and extractable organic carbon (C_{hws}) were determined by segmental flow-analysis using the colorimetric method on a SKALAR^{plus}SYSTEM (Skalar, Netherlands).

0.01M CaCl₂ extraction

Fresh soil was used for assessment of mineral N [$N_{min(CaCl_2)}$] and total extractable nitrogen (N_{CaCl_2}). The extraction agent 0.01M CaCl₂ (Houba et al. 1986) was used (1:10, w/v). Contents of total extractable nitrogen (N_{CaCl_2}), $N-NO_3^-$, $N-NH_4^+$ con-

tents and extractable organic carbon (C_{DOC}) were determined using segmental flow-analysis using a colorimetric method on a SKALAR^{plus}SYSTEM (Skalar, Netherlands).

Contents of total nitrogen N_t and carbon C_t were determined using oxidation on a LECO CNS 2000 (LECO Corporation, USA). For soil analyses soils were sampled after the harvest of barley in 2003, 2004 and 2005.

RESULTS AND DISCUSSION

Table 4 shows N balance in both experiments. The inputs included N from applied fertilizers while outputs included N removals by the cultivated crops during the growing periods 1997–2005. The balance was calculated as a difference between inputs and outputs. Atmospheric depositions are not included in the N balance (dry and wet deposition, N from biological fixation). Wet depositions can account for 9 kg N/ha/year (Balík et al. 1995). Negative N balance was found for the non-fertilized control treatments of both experiments and for Cambisol fertilized only by mineral N. The application of organic materials (sewage sludge and manure) and combination of mineral N fertilizer and barley straw (N + straw) resulted in a positive N balance.

Table 4. Balance of nitrogen in regions Suchdol (Chernozem) and Lukavec (Cambisol) in the years 1997–2005 (kg/ha)

Number of treatment	Treatment	N amount applied during 9 years	Removal during 9 years		Balance (input-output)	
			Chernozem	Cambisol	Chernozem	Cambisol
1	control	0	668	591	–668	–591
2	SS1	990	828	822	+162	+168
3	SS3	2970	1029	1050	+1941	+1920
4	FYM	990	692	761	+298	+229
5	N	990	939	1065	+51	–75
6	N + straw	1071	939	1060	+132	+11

SS – sewage sludge; FYM – farmyard manure

Table 5. Total contents of C_t and N_t in 2005 (V%)

Number of treatment	Treatment	Chernozem		Cambisol	
		C_t	N_t	C_t	N_t
1	control	2.458	0.256	1.427	0.236
2	SS1	2.478	0.236	1.653	0.247
3	SS3	2.517	0.249	1.821	0.261
4	FYM	2.595	0.244	1.886	0.257
5	N	2.510	0.244	1.605	0.238
6	N + straw	2.385	0.228	1.610	0.238

SS – sewage sludge; FYM – farmyard manure

Significantly positive N balance of treatment SS3 in both regions could indicate a high danger of N losses by denitrification or leaching since the comparison of yields of this treatment and yields from other treatments did not show significant differences and therefore mineralized N was not bound by crop biomass.

In the case of Chernozem, 4.66 t C/ha was applied with FYM, 2.40 t C/ha was applied for SS1 treatment and 7.20 t C/ha was applied for treatment SS3. Straw was applied for treatment 6 at the rate of 2.32 t C/ha.

Total contents of C_t and N_t in Chernozem and Cambisol are shown in Table 5. Higher content of SOM was measured in Chernozem in contrast to the Cambisol. On the other hand, N contents in both regions were only slightly different. The duration of both experiments (9 years) is too short to confirm the effect of mineral N fertilizing (treatment 5) on C content in soil as was confirmed by the results of Balík et al. (2003). Also Collins et al. (1992) concluded that application of mineral N fertilizers increased the mineralization of SOM. In our experiments, the highest contents of C_t in both regions were measured in the treatment with FYM (treatment 4), which is in a good agreement with the amount and quality of the applied organic compounds of this fertilizer. Our results confirmed lower contents of C_t in the treatment with straw compared to the application of FYM and sewage sludge treatments. The mineralization of supplied organic matter is more intensive compared to treatments with sewage sludge and FYM. Our results clearly showed that C and N compounds are less stable in Cambisol, which leads to a higher rate of mineralization.

Table 6 shows a portion of extractable N_{hws} and N_{CaCl_2} from the total content of N_t and a portion of C_{hws} and C_{DOC} from the total content of C_t as

averages from the observed treatments. In the Chernozem, N_{hws} formed 3.66% from the total N content in the soil whereas N_{CaCl_2} formed only 0.82%. C_{hws} formed 2.98% and C_{DOC} 0.34% from the total C content. Cambisol contains 4.81% of N_{hws} and 0.84% of N_{CaCl_2} from the total N amount and 5.76% of C_{hws} and 0.70% of C_{DOC} from the total C content. After using these results in calculations of the topsoil weight per hectare (depth of arable soil was 25 cm), we obtained the following values: in Chernozem, hot water extraction released 333 kg N/ha and 3186 kg C/ha, whereas 75 kg N/ha and 320 kg C/ha was extracted by the 0.01M $CaCl_2$ solution. In Cambisol, hot water extracted 444 kg N/ha and 9220 kg C/ha, whereas only 78 kg N/ha and 436 kg C/ha was extracted by 0.01M $CaCl_2$. These results confirmed higher mineralization of the organic matter in the Cambisol. Similar results were published by Kubát et al. (2004) who investigated contents of total and decomposable carbon in more than 140 localities in the Czech Republic.

Table 6 clearly shows that hot water extraction is a stronger extraction method compared to the 0.01M $CaCl_2$ extraction. Data from Tables 7 and 8 confirmed this comparison. Hot water extraction extracts extinct soil microbial biomass, simple organic compounds and also organic compounds hydrolyzed and depolymerized. Extraction using 0.01M $CaCl_2$ released only ammonium and

Table 6. Portion (%) of N_{hws} and N_{CaCl_2} from total contents of N_t ; portion (%) of C_{hws} and C_{DOC} from total contents of C_t (average from observed treatments)

Soil	N_{hws}	N_{CaCl_2}	C_{hws}	C_{DOC}
Chernozem	3.66	0.82	2.98	0.34
Cambisol	4.81	0.84	5.76	0.70

Table 7. Hot water extraction of Chernozem and Cambisol samples (averages for the years 2003–2005 in ppm)

Number of treatment	Treatment	Chernozem				Cambisol			
		N _{min(hws)}	N _{org(hws)}	N _{hws}	C _{hws}	N _{min(hws)}	N _{org(hws)}	N _{hws}	C _{hws}
1	control	29.1	64.9	94,1	696.3	22.5	79.4	101.9	808.5
2	SS1	35.4	68.1	103,6	697.6	29.5	86.7	116.3	947.9
3	SS3	36.2	78.3	114,5	698.8	35.1	96.4	131.5	1079.4
4	FYM	36.3	78.8	115,1	852.3	38.2	97.3	135.4	1097.7
5	N	35.3	80.7	116,1	774.6	33.5	84.1	117.6	912.7
6	N + straw	35.1	71.8	106,9	725.3	30.2	77.2	107.4	918.3

SS – sewage sludge; FYM – farmyard manure

nitrate ions (also nitrite ions) to the soil solution and form the soil sorption complex together with very easily soluble organic carbon and nitrogen. The highest contents of fractions extracted by hot water were measured in the treatment with manure of Cambisol: 38.2 ppm N_{min(hws)} and 97.3 ppm N_{org(hws)}, 135.4 ppm N_{hws} [N_{min(hws)} + N_{org(hws)}] and 1097 ppm C_{hws}. After the extraction using 0.01M CaCl₂, the highest content of N_{min(CaCl₂)} was determined in the treatment SS3 (19.6 ppm) of Cambisol. The highest content of N_{CaCl₂} [N_{min(CaCl₂)} + N_{org(CaCl₂)}] was recorded in the treatment SS1 of Cambisol as well as the content of C_{DOC} 135.8 ppm.

This is an evidence of a strong effect of soil type on mineralization of N and C from the comparison of measured data of both experiments with differing soil-climate conditions. These results were confirmed by Garau et al. (1986) who found that soil type was the most important factor affecting N mineralization. The second dominant factor was the amount and properties of the applied fertilizers. In agreement with the results

of Meng et al. (2005), we confirmed that the application of manure increased dramatically the content of soil organic N and C compared to the non-fertilized control treatment. Soil organic matter, represented here as the C_{hws} content, is more rapidly decomposed after the application of sewage sludge compared to manure application (Iakimenko et al. 1996). According to Kaur et al. (2008), we found that contents of extractable C increased with increasing application of organic fertilizers. Contents of N_{min(hws)}, N_{org(hws)} and N_{hws} were lower in treatments fertilized by sewage sludge compared to manure. Comparison of treatment 5 (treatment N) and treatments with sludge (SS1, SS3) of Cambisol showed lower contents of measured fractions of N and C extracted by both hot water and 0.01M CaCl₂. However, higher amount of N_{org}, N_{hws} and C_{hws} was determined by hot water extraction in treatment 5 (treatment N) of Chernozem. This is probably caused by more favorable soil-climatic conditions in the Suchdol region that positively influenced growth and yield of crops and also the amount of crop residues (root

Table 8. Extraction of Chernozem and Cambisol samples by 0.01M CaCl₂ (averages for the years 2003–2005 in ppm)

Number of treatment	Treatment	Chernozem				Cambisol			
		N _{min(CaCl₂)}	N _{org(CaCl₂)}	N _{CaCl₂}	C _{DOC}	N _{min(CaCl₂)}	N _{org(CaCl₂)}	N _{CaCl₂}	C _{DOC}
1	control	11.4	3.8	14.1	81.9	10.1	6.3	14.4	81.1
2	SS1	18.9	4.8	27.6	82.0	18.9	9.1	22.4	135.8
3	SS3	18.3	4.0	21.9	85.1	19.6	6.6	22.4	128.3
4	FYM	15.7	3.9	18.7	103.7	13.0	6.7	20.4	110.2
5	N	13.4	3.6	14.3	70.8	12.7	6.2	20.9	107.8
6	N + straw	14.7	3.8	22.9	88.5	17.1	7.2	23.6	125.8

SS – sewage sludge; FYM – farmyard manure

Table 9. C/N ratio in soils for C_t and N_t , hot water extraction and 0.01M $CaCl_2$ extraction in 2005

Number of treatment	Treatment	Chernozem			Cambisol		
		C_t/N_t	C_{hws}/N_{hws}	C_{DOC}/N_{CaCl_2}	C_t/N_t	C_{hws}/N_{hws}	C_{DOC}/N_{CaCl_2}
1	control	9.6	8.2	5.8	6.1	7.9	6.2
2	SS1	10.5	8.3	3.0	6.7	8.2	6.1
3	SS3	10.1	7.8	3.9	7.0	8.2	5.7
4	FYM	10.6	9.2	5.5	7.4	8.1	5.4
5	N	10.3	7.8	5.0	6.8	7.8	5.1
6	N + straw	10.5	9.0	3.9	6.8	8.5	5.3

SS – sewage sludge; FYM – farmyard manure

hairs, roots, stubble). Crop residues remained in the soil and influenced contents of the mentioned C and N fractions extractable mainly by hot water.

Table 9 demonstrates the comparison of C/N ratios measured by different extraction methods. According to the used extraction method, the C/N ratios varied dramatically. The lowest ratios were obtained after the $CaCl_2$ extraction where soluble organic carbon was only extracted and thus the C_{DOC}/N_{CaCl_2} ratio is low. Hot water extracted carbon from the biomass, simple organic compounds and depolymerized and hydrolyzed compounds. The lowest C_t/N_t ratio in both experiments was found in the control treatment. The C_t/N_t ratio in Chernozem was 9.6:1 and in Cambisol 6.1:1. From the comparison of other treatments, it is evident that the C_t/N_t ratio did not fluctuate in dependence on N fertilizing treatment. When hot water extraction method was used, identically in both experiments, the lowest C_{hws}/N_{hws} ratio was determined in N treatment N (7.8:1), and also in treatment SS3 of Chernozem. C_{hws}/N_{hws} ratio was found in the interval of 7.8–9.2:1 in Chernozem and 7.8–8.5:1 in Cambisol. Landgraf et al. (2003) published similar results. According to these authors, the C_{hws}/N_{hws} ratio was determined in the interval of 7.0–11.0:1. When 0.01M $CaCl_2$ was used, the lowest $C_{DOC}:N_{CaCl_2}$ ratio was found in SS1 treatment in Chernozem (3.0:1), and in

N treatment in Cambisol (5.1:1). Landgraf et al. (2003) determined the C/N ratio using 0.05M KCl in the interval of 5.0–14:1.

Various systems of N fertilization strongly influenced the content and various forms of C and N in the soil and also the C/N ratio. After the comparison of the evaluated treatments, the best results were obtained in both experiments in manure treatment. The application of manure gave the highest amounts of high-quality organic compounds with a relatively high C/N ratio. Similar relations were also described by Sadej and Przekwas (2008).

Nitrogen extracted by 0.01M $CaCl_2$ formed only 22.4% of N extractable by hot water in Chernozem and 17.5% in Cambisol (Tables 7 and 8). A high portion of organic N compounds besides mineral N was extracted by hot water (Table 7). After extraction by 0.01M $CaCl_2$ mineral N was predominantly extracted (Table 8). Hot water extraction method provides long-term information on N mineralization in the soil whereas extraction by 0.01M $CaCl_2$ represents actual data of the content of easily mineralizable N compounds.

It was not possible to calculate the analysis of variance for different soil-climate conditions in selected regions, therefore a linear model of regression analysis was used for followed relationships.

A statistically significant relationship between extracted C and N forms and total content of C_t

Table 10. Relationships between extracted forms of N and total content of nitrogen in soil

Soil	Chernozem			Cambisol		
	r^2	R^2	level of significance (α)	r^2	R^2	level of significance (α)
N_{hws}	0.39	15%	NS	0.91	83%	0.05
N_{CaCl_2}	-0.65	42%	NS	0.36	13%	NS

NS – not significant

Table 11. Relationships between extracted forms of C and total content of carbon in soil

Soil	Chernozem			Cambisol		
	r^2	R^2	level of significance (α)	r^2	R^2	level of significance (α)
C_{hws}	0.68	46%	NS	0.99	99%	0.01
C_{DOC}	0.38	15%	NS	0.48	23%	NS

NS – not significant

Table 12. Relationships between content of extracted forms of soil N and content of C

Method	Hot water extraction			Extraction by 0.01M $CaCl_2$		
	r^2	R^2	level of significance (α)	r^2	R^2	level of significance (α)
Chernozem	0.60	36%	NS	0.20	4%	NS
Cambisol	0.96	93%	0.01	0.98	95%	0.01

NS – not significant

and N_t was found only in Cambisol after hot water extraction. Relationship between content of N_{hws} and N_t (Table 10) is described by the following equation:

$$N_{hws} = -152.88 + 0.11 \times N_t \quad (1)$$

Strong correlation is proved by the correlation coefficient $r^2 = 0.91$ and determination coefficient $R^2 = 82\%$ with the level of confidence 95%. Landgraf et al. (2003) found also a strong correlation between N_{hws} and N_t , with a correlation coefficient $r^2 = 0.89$ with the level of confidence higher than 99%.

Relationship between content of C_{hws} and C_t (Table 11) is described by the following equation:

$$C_{hws} = -141.44 + 0.07 \times C_t \quad (2)$$

The coefficient of correlation $r^2 = 0.99$ and coefficient of determination $R^2 = 99\%$ with the level of confidence 99% in Cambisol proves a strong correlation. Similar results were published by Cerhanová et al. (2007). Authors determined a positive correlation between C_{hws} and C_t .

Strong relationship between content of N_{hws} and C_{hws} was found in Cambisol (Table 12). Coefficient of correlation $r^2 = 0.96$ and coefficient of determination $R^2 = 93\%$ proves a strong correlation. Meng et al. (2005) and Friedlová et al. (2007) showed similar results.

$$N_{hws} = 7.96 + 0.11 \times C_{hws} \quad (3)$$

A statistically significant relationship between N_{CaCl_2} and C_{DOC} was found. Coefficient of correlation $r^2 = 0.98$ and coefficient of determination $R^2 = 95\%$ shows that content of N_{CaCl_2} was in strong relation with content of C_{DOC} .

$$N_{CaCl_2} = -8.39 + 0.26 \times C_{DOC} \quad (4)$$

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