

Soil carbon transformation in long-term field experiments with different fertilization treatments

JIŘÍ BALÍK*, JINDŘICH ČERNÝ, MARTIN KULHÁNEK, ONDŘEJ SEDLÁŘ

Faculty of Agrobiological, Food and Natural Resources, Czech University of Life Sciences Prague, Prague, Czech Republic

**Corresponding author: balik@af.czu.cz*

ABSTRACT

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Soil carbon transformation was observed in long-term stationary field experiments (longer than 20 years) at two sites with different soil-climatic conditions (Luvisol, Chernozem). The following crops were rotated within the trial: row crops (potatoes or maize)-winter wheat-spring barley. All three crops were grown each year. Four different fertilization treatments were used: (a) no fertilizer (control); (b) sewage sludge (9.383 t dry matter/ha/3 years); (c) farmyard manure (15.818 t dry matter/ha/3 years); (d) mineral NPK fertilization (330 kg N, 90 kg P, 300 kg K/ha/3 years). At the Luvisol site, the control treatment showed a tendency to decrease organic carbon (C_{org}) in topsoil. At organic fertilization treatments the content of C_{org} increased: sewage sludge – +15.0% (Luvisol) and +21.8% (Chernozem), farmyard manure – +19.0% (Luvisol) and +15.9% (Chernozem). At the NPK fertilization, the increase was +4.8% (Luvisol) and +4.7% (Chernozem). The increased C_{org} content was also associated with an increase of microbial biomass carbon (C_{mic}) and extractable organic carbon (0.01 mol/L $CaCl_2$ and hot water extraction). The ratio of C_{mic} in C_{org} was within the range 0.93–1.37%.

Keywords: farmyard manure; microbial biomass; organic matter; sewage sludge

Soil fertility care comprises mainly maintenance of soil organic matter; that is not only its amount but especially its quality. Soil organic matter is a key factor determining biological, chemical and physical properties of soils. Among the most important carbon (C) sources there are plant roots and post-harvest residues, as well as traditional organic fertilizers such as farmyard manure, slurry and compost. An alternative source of carbon in soil may be sewage sludges. In past, they contained high amounts of risk elements and negative impacts of sludges on soil quality were recorded. Brookes and McGrath (1984) reported a 50% decrease of soil microbial biomass in treatments with sludges as compared to manure. Smith (1991) assigned a

soil microbial biomass decrease definitely to the negative impact of risk element in sludges. The content of microbial biomass carbon (C_{mic}) is one of the important indicators of soil organic matter transformation (Brookes et al. 1985, Mueller et al. 1998). Soil organic matter (SOM) consists of primary organic matter (POM) and humus. According to Körschens et al. (1990) SOM may be divided into 2 fractions. One is inert (stable) and closely correlates with the content of clay particles, the other fraction is potentially mineralizable and it may be denominated as active organic carbon. For its extraction, Körschens et al. (1990) used hot water (hot water soluble carbon – C_{HWS}). A very easily mineralizable fraction is 0.01 mol/L

CaCl₂-extractable organic carbon (C_{DOC}) (Nedvěď et al. 2008). To study soil carbon transformation is very difficult and it requires long-term field experiments.

The aim of this study was to determine changes in the content of soil organic matter and extractable carbon fractions in soils at different organic and mineral fertilization treatments at sites with different soil-climatic conditions.

MATERIAL AND METHODS

The effect of fertilization on grain yield of winter wheat and spring barley was observed in precise long-term field trials. These trials were established in 1996 at two sites of the Czech Republic with different soil-climatic conditions: Červený Újezd and Praha-Suchdol (Table 1). Within the trials, three crops were rotated in the following order: potatoes, winter wheat, spring barley. Because of the agrotechnical conditions of the Červený Újezd site, potatoes as the experimental crop were replaced by silage maize. The trial comprised 4 treatments: (1) no fertilization (control); (2) sewage sludge (SS); (3) farmyard manure (FYM); (4) NPK in mineral fertilizers (NPK). Organic fertilizers – sewage sludge and farmyard manure – were always applied in autumn (October) to potatoes (maize). Mineral phosphorus and potassium fertilizers were applied to each crop in autumn; mineral nitrogen fertilizers were applied to potatoes (maize) and spring barley in spring prior to the crop establishment. In the case of winter wheat, the nitrogen dose was divided into halves; the first one was applied as regenerative fertilization, the second one as productive fertilization (Table 2). The dose of

Table 1. Experimental sites characteristics

Site	Červený Újezd	Suchdol
Location	50°4'22"N, 50°7'40"E, 14°10'19"E 14°22'33"E	
Altitude (m a.s.l.)	410	286
Mean annual temperature (°C)	7.7	9.1
Mean annual precipitation (mm)	493	495
Soil type	Luvisol	Chernozem
Soil texture	loam	loam
pH _{CaCl₂}	6.5	7.5
Organic carbon (%)	1.168	1.486
Cation exchange capacity (mmol ₊ /kg)	145	230
P	100	91
K	80	230
Mg	110	240
Ca	3600	9000

nitrogen was 140 kg N/ha for wheat and 70 kg N/ha for spring barley. The NPK treatment of winter wheat and spring barley included phosphorus at a rate of 30 kg P/ha (triple super phosphate) and potassium at a rate of 100 kg K/ha (60% potassium chloride). Average characteristics of organic fertilizers and their dry matter application rates are shown in Table 3. The sewage sludge used for application at both experimental sites originated from one wastewater treatment plant during all experimental years.

During the 3 years (2014/2016), soil samples (depth 30 cm) were taken for determination of contents of organic carbon, microbial biomass carbon and other extractable forms of carbon. The monitoring was always carried out on blocks of wheat and potatoes (maize). Soils were sam-

Table 2. Application rates of nutrients (kg/ha) – (3-year cycle)

Treatment	Potatoes/maize			Wheat			Barley		
	N	P	K	N	P	K	N	P	K
Control	–	–	–	–	–	–	–	–	–
Sewage sludge	330 ¹	201 ²	55 ²	0	0	0	0	0	0
Farmyard manure	330 ¹	118 ²	374 ²	0	0	0	0	0	0
NPK ³	120	30	100	140	30	100	70	30	100

¹Nitrogen as the total nitrogen in organic fertilizers; ²Average yearly dose depends on the nutrient content in organic fertilizers (Table 3); ³Mineral fertilizers: N – calcium ammonium nitrate (27% N); P – triple super phosphate (21% P); K – potassium chloride (50% K)

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Table 3. Average characteristics of organic fertilizers and their dry matter (DM) application rates

	Dose (t/ha/3 year)	DM content (%)	Nitrogen content (% DM)	Carbon content (% DM)
Sewage sludge	9.383	31.08	3.52	25.75
Farmyard manure	15.818	30.76	2.09	27.86

pled in each crop since the beginning of spring (March/April) in intervals of 2–3 weeks, and the last samples were taken after the harvest of the crop. Eight soil samplings were taken for each crop during one experimental year.

Extractable organic carbon

0.01 mol/L CaCl_2 extraction. The extraction agent 0.01 mol/L CaCl_2 was used (1:10, w/v). The content of C_{DOC} was determined in fresh soil samples by segmental flow-analysis using the infrared detection on a Skalar^{plus}System (Skalar, Breda, Netherlands).

Hot water extraction. Extraction using hot water (Körschens et al. 1990) was used for assessment of 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 C_{HWS} was determined by a segmental flow analysis using the infrared detection on a Skalar^{plus}System (Skalar, Breda, Netherlands).

Microbial biomass C. Microbial biomass C was estimated in fresh soil samples by the fumigation-extraction method after pre-extraction (Brookes et al. 1985, Mueller et al. 1998). It was calculated as a difference in C content in fumigated and non-fumigated sample (E_C) using the k_{EC} coefficient

(microbial biomass $C = E_C : k_{EC}$). The value of $k_{EC} = 0.45$ was used to calculate microbial biomass C (Mueller et al. 1998).

Total organic carbon. The content of total organic carbon in dry samples of soils, in farmyard manure and in sewage sludge was determined using oxidation on a carbon/nitrogen/sulphur analyser (Elementar Vario Macro, Elementar Analysensysteme, Hanau-Frankfurt am Main, Germany).

Statistical analysis. The results were assessed using the ANOVA statistical analysis. To evaluate the obtained results, the Statistica programme (StatSoft, Tulsa, USA) was used.

RESULTS AND DISCUSSION

The amount of plant biomass affects the individual carbon fractions in the soil, therefore the results of the average yields of crops grown on both sites were evaluated. Overall yield results are shown in Figure 1. To evaluate the results, values for all crops including the yield of straw at cereals obtained for the whole trial period of 20 years were related to the control treatment (control = 100%). Of course, there were significant differences among individual years; the results were influenced by weather conditions in individual

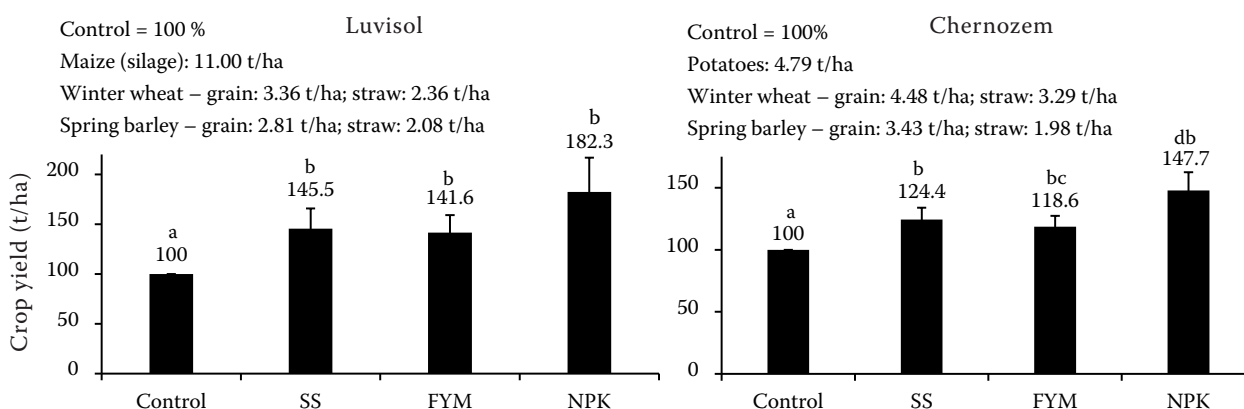


Figure 1. The effect of fertilization on crop yields during 1996–2016 period (100% dry matter). Control – no fertilization; SS – sewage sludge; FYM – farmyard manure; NPK – mineral fertilizers. Values with the same letters are not significantly different at $P < 0.05$

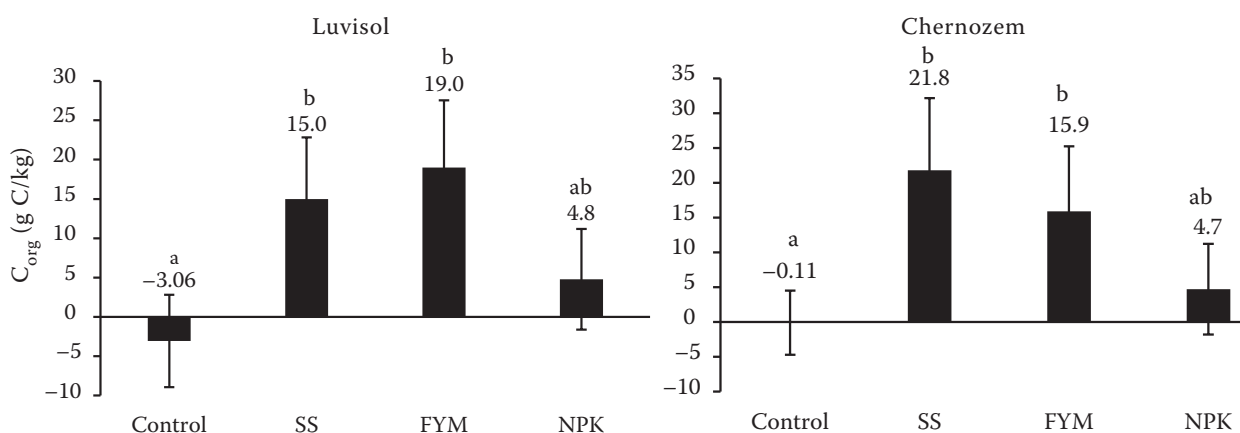


Figure 2. The relative changes of organic carbon (C_{org}) contents in soils (%) during 1996–2016 period. Control – no fertilization; SS – sewage sludge; FYM – farmyard manure; NPK – mineral fertilizers. Values with the same letters are not significantly different at $P < 0.05$

years, which increased variability of the whole data set. Significantly higher yields were obtained in mineral NPK fertilization treatments. The total N dose for a 3-year period (at crop rotation) was the same at all fertilization treatments (330 kg N/ha). Yet, at the NPK treatment a positive influence of relatively even distribution to individual crops was observed, which resulted in increased yields especially in barley and partly wheat. Organically fertilized plots did not vary significantly, however, at sludge treatment a tendency to increase yields was observed. Fertilization efficiency was higher at less fertile Luvisol sites, compared to Chernozem. Soil fertility potential at both sites could not be fully utilized due to the lack of precipitation during the growing period. The differences between control and other fertilization treatments were more pronounced with increasing length of the experimental period, mainly at Luvisols. In the first ten years (1996/2006) the average increase at fertilized plots at both sites was 133.1% compared to control; in 2007/2016 it was 153.6%. The ratio of grain and straw in wheat was 1:0.70 (Luvisol) and 1:0.73 (Chernozem), whereas in barley it was 1:0.74 (Luvisol) and 1:0.58 (Chernozem).

Organic carbon content. Changes in the organic carbon content in the topsoil are shown in Figure 2. The changes were calculated in relation to the initial state as follows:

$$\Delta C_{org} = [(C_{org,n} - C_{org,i}) / C_{org,i}] \times 100$$

Where: $C_{org,n}$ – total C_{org} (g C/kg) at our sampling time (2014/2016; $C_{org,i}$ – initial C_{org} content in 1996 (Johnson et al. 2014).

This evaluation was done at plots with wheat, potatoes and maize and it represents average values for a 3-year period.

The figures show a decrease of C_{org} content at the control plots, by 3.06% at Luvisol and by 0.12% at Chernozem. The crop rotation included 2 cereals and 1 row crop, i.e. crops with relatively low input of C compounds into soil. There was no perennial forage crop used in the experiment. The changes were more marked at less fertile Luvisols in contrast to Chernozem. The results also show that inappropriate fertilization systems may cause degradation even of fertile soils, such as Luvisol. Mineral fertilization treatment increased C_{org} content by 4.80% (Luvisol) and by 4.70% (Chernozem). Significantly higher yields of the aboveground biomass (Luvisol by 82.3%, Chernozem by 47.7%) and thus also higher amounts of plant roots and root exudates contributed to an increase of C_{org} in soil. As reported by Lynch and Wipps (1990), 30–60% of the net photosynthesis production is allocated in roots; out of this amount, a substantial part is released in form of organic compounds (rhizodeposition) and carbon dioxide into the rhizosphere. Out of the total amount of carbon accumulated in roots, rhizodeposition covers 4–70%. Körschens et al. (2013) evaluated long-term trials in Europe (with various lengths of 16–80 years) and observed an average increase of C_{org} in topsoil by 10% at mineral fertilization treatments. Lower values in our experiments are probably caused by a shorter trial length. Literature often brings contradictory data on the influence of mineral (mainly N) fertilization on the changes

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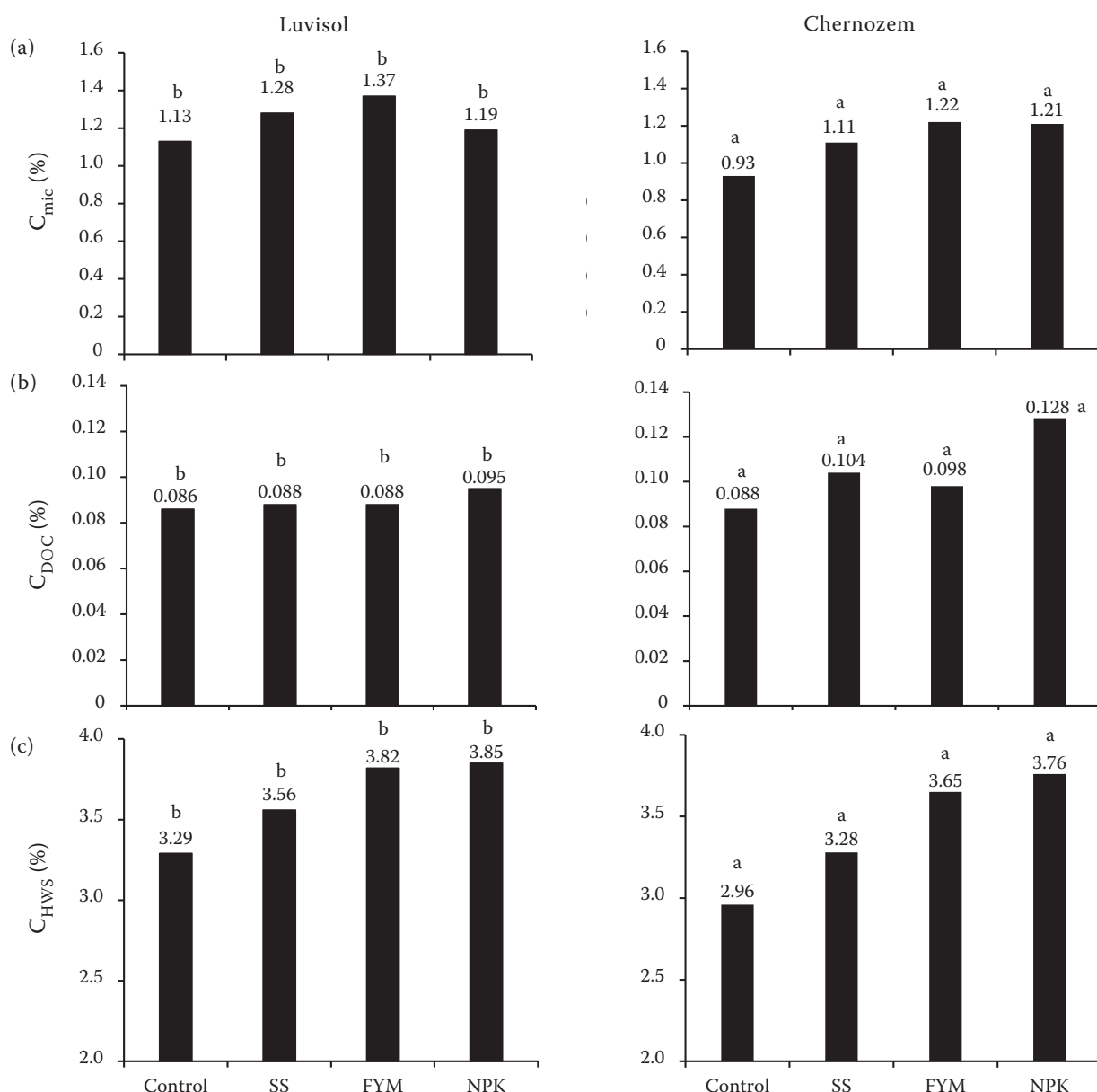


Figure 3. The portion of (a) microbial biomass carbon (C_{mic}); (b) $CaCl_2$ -extractable organic carbon (C_{DOC}) and (c) hot water soluble carbon (C_{HWS}) in organic carbon (C_{org}). Control – no fertilization; SS – sewage sludge; FYM – farmyard manure; NPK – mineral fertilizers. Values with the same letters are not significantly different at $P < 0.05$

of C_{org} content in the soil. This is caused by site-specific soil-climatic conditions (e.g. pH value), crops grown, and form and dosage of N fertilization. For instance, Shahbaz et al. (2017) reported a C_{org} increase by 12–14% compared to the initial state at treatments with intensive nitrogen fertilization in long-term trials (32 years).

Organic fertilization treatments in this experiment resulted in a significant increase of C_{org} content in topsoil. The average dose of fresh manure was 51.42 t/ha/3 years, which corresponds

to the agronomic recommendations for a sowing method of this type. Precise annual calculations suggest that in the experimental period, 30 850 kg C/ha was supplied into soil. At comparison with the carbon content in topsoil (depth 30 cm), carbon supplied by farmyard manure stands for 58.7% of the total amount of carbon in topsoil (Luvisol) and 46.1% (Chernozem). The increase of C_{org} in topsoil by 19% (Luvisol) and by 15.9% (Chernozem) thus corresponds to the fertilization intensity. Besides the organic fertilization, the increase is supported

also by higher yield of biomass yield, including roots and root exudates. The absence of mineral fertilization and thus lower mineralization of soil organic matter might also have played a role.

Sewage sludge rate used in the experiment significantly exceeds the limits set for the sludge application on the agricultural soils in the Czech legislation. The allowed rate is 5 t dry matter/ha/3 years. This limit was surpassed almost 2 times. Meanwhile, the supplied amount of carbon was significantly lower compared to manure and totalled 16 910 kg C/ha. Compared to the carbon content in the topsoil it was 32.1% (Luvisol) and 25.3% (Chernozem). Although the rate of carbon supplied by sewage sludge was lower compared to manure, the changes in the C_{org} content in topsoil were at the same level, in case of sludge at Chernozem even higher. This might be due to supplied sludge mineralization or an increase of the root biomass, which corresponds to the fact that in the sludge treatment the yields of above-ground biomass were higher compared to the manure treatment.

Microbial biomass carbon content. In a 3-year period, the content of microbial biomass C (C_{mic}) was observed. Figure 3a shows relations (in %) to total C_{org} . The ratio of C_{mic} in our experiments was 0.93–1.37% and it was lower compared to Scherer et al. (2011), who reported 1.7–3.3% in long-term trials with sludge, manure and composts. Fierer et al. (2009) published even lower values than those in our study, namely 0.6–1.1%. On the contrary, Rasmussen and Collins (1991) reported the values of 2.3–2.9%. All these values show the influence of specific soil-climatic conditions as well as other factors, such as crop rotation, mineral and organic fertilization intensity, etc. Median values at control were 121 mg C_{mic} /kg (Luvisol) and 149 mg C_{mic} /kg (Chernozem) – Figure 4a,b. Also at other treatments, the C_{mic} values at the Luvisol site were by 15–25% lower compared to Chernozem, which corresponds to naturally lower soil fertility of Luvisol. The highest values were reported after the application of manure (Figure 4a,b). Černý et al. (2008) reported the C_{mic} values obtained in the same experiment on Chernozem for a period from its establishment (1996) till 2005. The authors determined a difference between control and sludge/manure treatment as 11% and 12%, respectively. Differences obtained in our experiment are more significant 21.5% (sludge) and 18.1% (manure).

It confirms the fact that even at fertile sites, soil fertility degradation may progress if the soils are left unfertilized in the long term or unbalanced crop rotation is used. As reported by Fliessbach et al. (2007), high input of organic matter into soil results in an increased content of C_{mic} . Our data confirm these results for sludge and manure at both sites. The ratio of C_{mic} in C_{org} is higher at the farmyard manure application compared to sewage sludge (Figure 3a). Similarly, Scherer et al. (2011) also reported a significantly higher C_{mic} ratio at the manure treatment than at sludge. Compared to our trials, in their experiments with sludge significantly higher carbon amounts were supplied than with manure. Sewage sludge in our experiment was the same throughout the whole trial period and it contained significantly lower concentrations of risk elements than the limits set by legislation. At the same time, the amount of sludge used significantly overpassed the permissible dosage. However, neither a significant increase of the total risk elements concentrations at the sludge treatment, nor their concentrations in mobile soil fractions were recorded; hence, it may be presumed that their activity did not affect the C_{mic} content in soil. It must be also highlighted that at the sludge treatment, significantly less carbon was supplied compared to manure, which is probably the main reason of differing content of C_{mic} . Similarly, Johansson et al. (1999) did not observe a negative effect of high sludge rates application (8 t dry matter/ha/2 years) on soil microorganisms in the long-term trials.

The NPK values approximately by 10% higher than control confirm a positive effect of mineral fertilization (Figure 4a,b). It is interesting that the 10% difference is in good agreement with the results obtained by Geisseler and Scow (2014), who evaluated 64 long-term trials all over the world (107 data sets) and found an average increase of C_{mic} by 15.1% at treatments with mineral nitrogen fertilization compared to the control. Significance of the effect was related to the pH change. If the pH decreased to ≤ 5 no positive effect was observed whereas at higher values it was almost always positive. The positive effect was in correlation with the length of the trials, especially if the trial period was longer than 20 years. In our experiment, the value of pH_{CaCl_2} at the NPK treatment was 6.16 (Luvisol) and 7.23 (Chernozem). Also Fierer and Jackson (2006) in their extensive studies confirm a significant influence of soil reaction on the amount,

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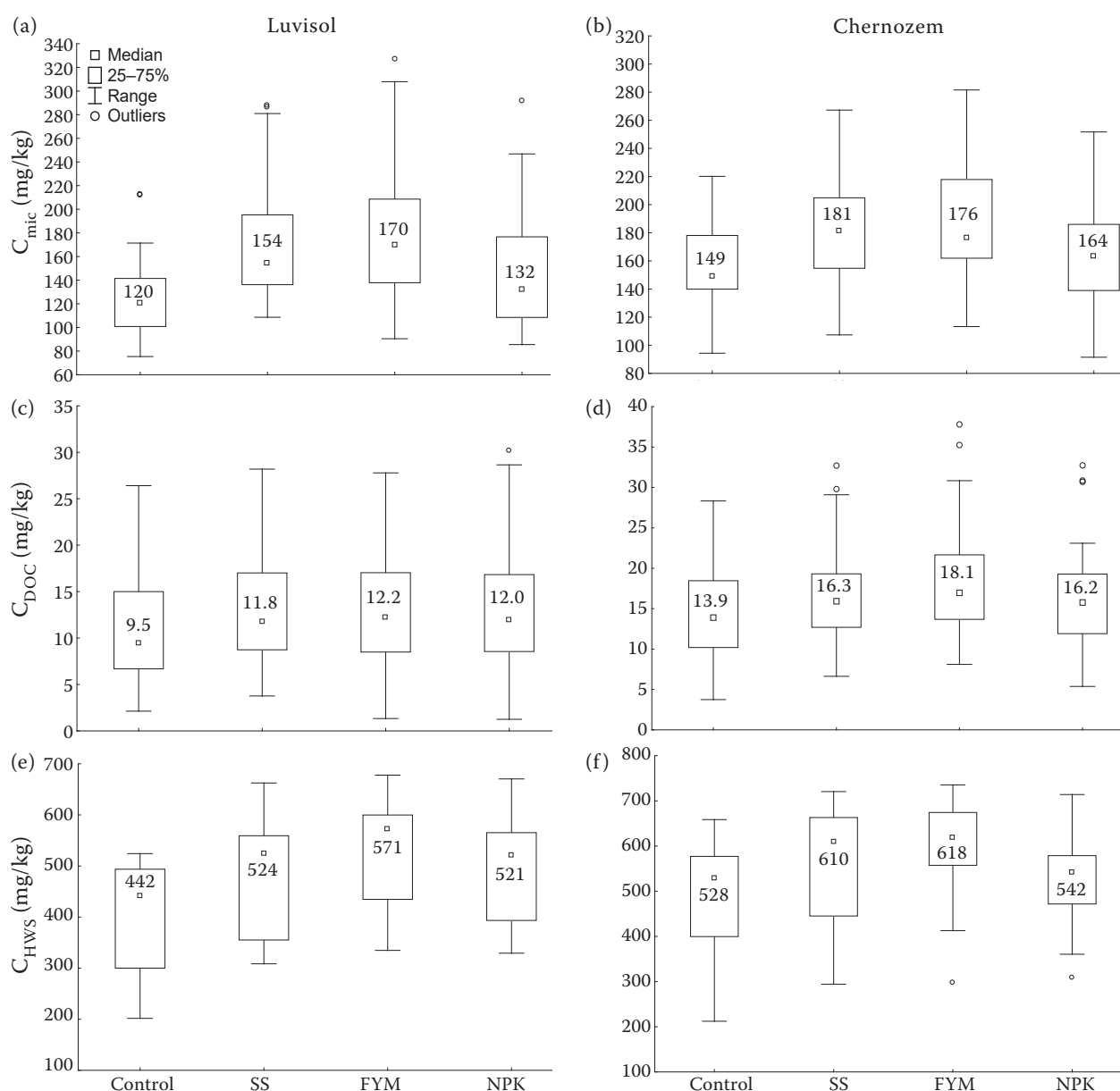


Figure 4. The content of (a,b) microbial biomass carbon (C_{mic}) (mg/kg); (c,d) $CaCl_2$ -extractable organic carbon (C_{DOC}) and (e,f) hot water soluble carbon (C_{HWS}) Control – no fertilization; SS – sewage sludge; FYM – farmyard manure; NPK – mineral fertilizers

composition and activity of soil microbial biomass. Differences were observed especially at $pH < 5$. Also in the experiments carried out by Kallenbach and Grandy (2011) the content of C_{mic} at fertilization treatments increased by 9% compared to control. Similarly, a C_{mic} increase was observed in long-term trials in Sweden (Ultuna) at treatment with calcium nitrate compared to control and ammonium sulphate treatment (Marstorp et al. 2000). A significant increase of the aboveground biomass growth at the NPK treatment positively

influenced the content of C_{mic} in our trials. It is supported also by the results of Börjesson et al. (2016) who studied the influence of cultivated crop on the content of C_{mic} . They found that after 13 years of maize monoculture, C_{mic} comprised 10–33% carbon from maize, i.e. from roots and root exudates. The authors further stated that this value was influenced by the level and quality of organic and mineral fertilization.

Extractable carbon fractions. A very weak extraction agent (0.01 mol/L $CaCl_2$) corresponds to

low values of the released carbon (Figure 4c,d). With respect to the treatment and site, it was 0.084% to 0.128% of C_{org} (Figure 3b). At hot water extraction, a slightly mineralizable fraction should be released (Körshens et al. 1990). Though this fraction has not been precisely described, yet, it is presumed that it comprises part of the microbial biomass and simple organic compounds hydrolysable in hot water (Schulz 1997). In hot water extraction, 2.96% to 3.85% of C_{org} were mobilized (Figure 3c). Median values are in intervals 442–571 mg C_{HWS} /kg (Luvisol) and 542–610 mg C_{HWS} /kg (Chernozem) (Figure 4e,f). Šimon et al. (2013) in their study reported values lower by ca. 1/3. In their experiments, in accordance with our results, significantly higher values were also obtained at the manure application and NPK fertilization. Bolinder et al. (2007) supposed that ca. 75% are root exudates and 25% is the ratio of stubble. Residues with low C:N ratio decompose faster than those with a higher ratio, for instance plant roots (Cotrufo et al. 2013). Roots thus contribute to more stable C_{org} fraction in soils. Based on the prerequisite of a smaller amount of root exudates and amount of roots at control, the ratio of stabile forms of organic substances should be the highest in this treatment. In our study, the lowest ratio of 0.01 mol/L CaCl_2 and hot water extractable C (C_{DOC}) (C_{HWS}) in C_{org} was recorded at both sites. Differences compared to other treatments are nevertheless small, which is due to a variability of plots at individual sites and relatively short trial period.

Root exudates are an important source of labile carbon for soil microorganisms (Luo et al. 2014). The chemical composition of exudates is as follows: sugars 50–70%, carboxylic acids 20–30%, amino acids 10–20% (de Graaff et al. 2010). Root exudates stimulate fast growth of soil microbes, which may lead to increased mineralization of original organic compounds, a so called priming effect (Merino et al. 2015). Intensive mineral fertilization may thus result in increased C_{org} in soil, but at the same time in an increase of more labile organic fractions (Shahbaz et al. 2017). Also clay fractions that sorb these low-molecular organic compounds have a great impact on the whole process. The results are thus influenced by specific soil-climatic conditions. In our experiments, the NPK treatment gives the highest ratio of C_{DOC} and C_{HWS} in C_{org} (Figure 3b,c); however, these differences are not significant. It is not possible to prove that the NPK fertilization

increases the ratio of mobile carbon fraction at the expense of stabile fractions, probably due to a short experimental period, relatively low intensity of nitrogen fertilization and quality of soils at experimental sites (Luvisol and Chernozem).

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