The Influence of Long-term Organic and Mineral Fertilization on Soil Organic Matter

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Abstract: Parameters evaluating soil organic matter quantity (organic C and N content) and quality (hot water extractable C content, aliphatic compounds, microbial biomass C content, basal respiration activity) were determined in soils differently fertilized (NPK – mineral fertilization 64.6 kg N/ha/year, FYM – farmyard manure 38.6 kg N/ha/year, FYM + NPK – 103.3 kg N/ha/year) in long-term field experiment established in 1955 in Prague. Variant without any fertilization was used as a control. Nine years crop rotation (45% cereals, 33% root crops, 22% fodder crops) is practiced in this long-term experiment. Soil samples were taken from the arable layer (0–20 cm) in spring over the period of 1994–2004. Continual application of FYM and FYM + NPK increased the organic carbon content, hot water extractable C (HWC) content, aliphatic compounds content and microbial biomass C content significantly compared to control variant. Mineral fertilization (NPK) increased only organic C content significantly compared to control variant; HWC content, aliphatic compounds content and microbial biomass C content were increased not significantly. Basal respiration activity did not differ significantly between the variants but the influence of plants cropped in individual years on the basal respiration was observed. The total N content was increased significantly only in FYM + NPK variant as compared to control variant. Presence of lucerne in crop rotation contributed positively to the total nitrogen content in soil of all variants due to the symbiotic N_2 fixation. C:N ratio varied from 9.96 to 10.46. Significant positive relationships (r = 0.30 to 0.68; $P < 0.05$) among the all parameters were determined with exception of basal respiration activity. The most of measured characteristics tended to be constant or slightly increase in the period of observation that shows evidence of stability of this soil management system.

Keywords: long-term field experiment; organic and mineral fertilization; crop rotation; soil organic matter

Agricultural management practices influence quantitatively and qualitatively soil organic matter (SOM). While conventional farming practice generally leads to a reduction of soil organic matter, organic management resulted in significant increases in total organic C and N, available-P, soil respiration, microbial biomass, and enzyme activities compared with those found under conventional management (Melero *et al.* 2006). Organic amendments increase the organic carbon and nitrogen content in the soils (Madejón *et al.* 2001) and the addition of large amounts of organic materials to the soil could potentially increase total soil C concentrations (Blair *et al.* 2006). On the other hand, inorganic fertilizers also increase carbon inputs indirectly through increased crop growth and thereby increased crop residues incorporated to the soil (Paustian *et al.* 1997). Besides the fertilization systems, the crop rotation systems are also the important factor influencing

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the crop production systems stability. Thus optimal balanced crop rotation involving forage legumes can improve organic carbon status in the soil. Soon *et al.* (2007) who tested the influence of 12 years of four crop sequences on the organic C pools of a Grey Luvisolic sandy loam soil found increased total organic C content in the top 15 cm of soil in the red clover rotation compared to either the pea or fallow rotation. It was demonstrated that the farming practices lead to changes in SOM content. However, these changes are difficult to detect in the short or medium time (Ghani *et al.* 1996). It is therefore necessary to use the short-term sensitive characteristics. Soil microbial biomass, and hot water extractable carbon (HWC) were the most sensitive indicators among the soil biochemical measurements considered to reflect the changes in the SOM caused by different soil management practices (Ghani *et al.* 1999, 2003). Besides the biochemical methods, modern effective analytical techniques exist, and non-destructive FTIR spectroscopy as the sensitive infrared technique is one of them. Its application for characterization of organic matter and humic substances in soils is presently widely used (Ellerbrock *et al.* 2005). The FTIR spectra of humic macromolecules contain a variety of bands that are diagnostic and could serve as valuable tool to characterize the principal classes of chemical groups of which SOM is comprised (Davis *et al.* 1999; Solomon *et al.* 2005). Besides analyses of extracted humic substances, FTIR technique can be directly used for the study of SOM composition in a bulk soil samples (Šimon 2007a). The aim of this study was to evaluate the quantitative (organic carbon and total nitrogen) and qualitative (aliphatic compounds, HWC, microbial biomass, basal respiration) changes of the SOM under nine years rotation (45% cereals, 33% root crops, 22% fodder crops) field management. Organic and mineral fertilized variants of the long-term field experiment established in Prague in 1955 were selected for evaluation. Archived soil samples (period 1994–2006) were spectroscopically analysed to compare the content of aliphatic compounds with content of organic C, HWC, total N, microbial biomass C and basal respiration activity in these samples.

MATERIAL AND METHODS

Soil samples. Soil samples were taken from the selected plots of the long-term field experiment in Prague-Ruzyně. This experiment was founded in 1955 with the aim to investigate the effect of various fertilization systems on the yields, nutrient uptake and the soil quality. Experiment is splited to four blocks differing in organic manuring and mineral fertilization. The plots are squares, 12 × 12 m, with 1.5 m protective stripes on all sides and 9 × 9 m harvest plots in the middle. The altitude of the site is about 352 m above the sea level, average annual temperature is 8.1°C and average annual precipitation 450 mm. Soil type is Orthic Luvisol, clay-loam, developed on dilluvial sediments mixed with loess. Depth of the arable A horizon range between 26 and 33 cm, followed by a less apparent illuvial horizon, which passes in the non-carbonaceous substrate. Clay content (particles < 0.002 mm) in the arable layer is 31.3%. Organic carbon content in the arable layer ranges between 1.1% and 1.4%. Its content in the upper layer of the illuvial horizon remains still relatively high (0.6 to 0.9%). Cation exchange capacity in the arable layer is about 20 to 22 meqv/100 g and it increases with the depth to 30–33 meqv/100 g. Soil reaction is neutral (pH_{KCl} is 6.8–7.1) in the whole

Table 1. Variants selected for evaluation

Crop rotation	Variants	Average N doses (kg N/ha/year)	Average P doses (kg P/ha/year)	Average K doses (kg K/ha/year)
Lucerne, lucerne, winter	nil (control)	0	0	0
wheat, sugar beet, spring bar-	NPK	64.6	24	109
ley, potatoes, winter wheat,	FYM	38.6	6.5	25
sugar beet, spring barley	FYM + NPK	103.3	30.5	134

profile (Kubát *et al.* 2003). Four variants (control – no fertilization, NPK – mineral fertilization, FYM – farmyard manure and FYM + NPK) have been selected for this study. Crop rotation and organic and mineral N fertilization are shown in Table 1. Soil samples have been taken from 0–20 cm top layer in all variants (three partial samples per variant were mixed together) in spring in the period of 1994–2006.

Analyses. The soil samples were sieved through 2 mm sieve. The fresh soil samples were analysed for basal respiration and microbial biomass. A part of soil samples were air-dried and analysed for organic carbon (C_{ox}), total N and hot water extractable C (HWC). Organic C (C_{ox}) content was determined by wet combustion according to Alten *et al.* (1935). HWC content was determined according to Schulz and Körschens (1998). Total nitrogen (N_t) was determined in air-dried soil samples on a LECO analyzer. Basal respiration CO_2-C evolved after 7 days incubation of soil samples in 25°C was determined as the amount of organic C released as CO_2 after absorption in NaOH and precipitation with $BaCl_2$ and was analysed by titration with standard HCl (Apfelthaler 1984). Soil microbial biomass C was determined by the fumigation-extraction method according to Vance *et al.* (1987). For Fourier transform infrared (FTIR) analysis, the soil sample (300 mg) was mixed with 900 mg KBr (FTIR grade 99%, Aldrich, Germany) and ground in agate mortar. The homogenous mixture was transferred to the diffuse reflectance cup (dia 12 mm) without any pressure and leveled with microscope glass slide. The FTIR spectra were measured on Thermo Nicolet Avatar 320 FTIR spectrometer equipped by Smart Diffuse Reflectance accessory (Nicolet, USA). Three FTIR spectra (absorption mode, KBr background, 256 scans, data spacing 1.929 cm^{-1}) were collected for each soil sample. The aliphatic C-H signal area of the samples (3000–2800 cm^{-1}) was integrated by the spectrometer software (Omnic, version 6a, Nicolet, USA). All data were processed by analysis of variance followed by the Tukey test that evaluate the significance of differences among the variants. Correlations between the individual characteristics were calculated (STATISTICA software).

RESULTS AND DISCUSSION

Organic carbon

Average values of C_{ox} in the soil samples taken from the four selected variants of long-term experiment in the period of 1994–2006 are shown in Table 2. Continual application of both the FYM and NPK before and in the observed period increased the organic C content significantly as compared to control variant. The C_{ox} content in the individual variants differed significantly among each other and ranged as follows: FYM + NPK > FYM > NPK > control. The highest amount of C_{ox} was determined in the variant FYM + NPK where the additional effect of the mineral fertilization to the organic manuring was recorded. Average amount of increased C_{ox} content in this variant was 2.47 mgC/g dry soil (20.0%) as compared to control variant. Single organic manuring (FYM) increased the average C_{ox} content by 1.69 mgC/g dry soil (13.7%) and single NPK fertilization in-

Table 2. Average values for characterised parameters (period 1994–2006)

Variant	Organic carbon (mg/g)	Hot water extractable carbon (mg/g)	Aliphatic compounds (intensity)	Microbial biomass C (μg/g)	Basal respiration activity (μg/g)	Total nitrogen (mg/g)	C:N
Control	12.33a[a]	0.252a	0.534a	168.88a	27.64NS	1.249a	9.96NS
NPK	13.34b	0.276ab	0.646ab	170.86a	34.23	1.350ab	9.97
FYM	14.03c	0.303b	0.794bc	189.47b	32.62	1.363ab	10.46
FYM + NPK	14.80d	0.336c	0.873c	214.34b	33.62	1.484b	10.07

[a]means within the column followed the same letter do not differ significantly as determined by Tukey multiple range test (P < 0.05); NS – non-significant differences

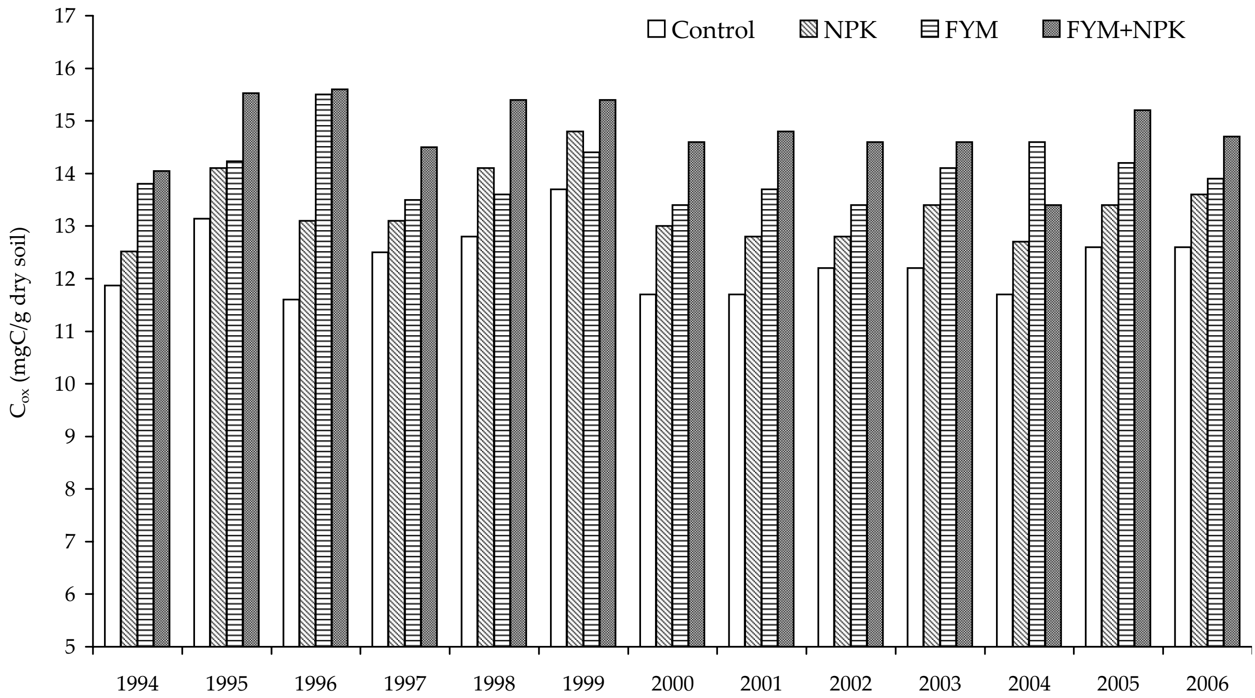

Figure 1. Organic carbon content (C_{ox}) in soil samples

creased the average C_{ox} content by 1.01 mgC/g dry soil (8.2%) as compared to unfertilized variant. Variation of the C_{ox} in individual variants in the period of observation is shown in Figure 1. C_{ox} remained approximately constant (FYM) or tended to slightly increase in the period of 1994–2006 (NPK, FYM + NPK, control) with the result that the differences among the variant were mostly saved for the whole period of observation. The effect of individual crops planted in individual year on the organic carbon content was registered but the differences among the individual values were not significant (Figure 1).

Hot water extractable carbon

Average values of HWC in the soil samples are shown in Table 2. Application of FYM + NPK resulted in the highest amount of this labile C fraction in this variant and significant differences among FYM + NPK and other variants were found. On the other hand single organic manuring (FYM) did not increase the HWC significantly as compared to the NPK variant but increased the HWC content significantly in comparison with control variant. NPK variant did not differ significantly from control variant. The differences among the individual variants in HWC content were similar to the organic C content, the range was as follows: FYM + NPK > FYM > NPK > control. Combined organic and mineral manuring (FYM + NPK) increased the average HWC content by 0.084 mgC/g dry soil (33.3%), single organic manuring (FYM) increased the average HWC content by 0.051 mgC/g dry soil (20.0%) and single NPK fertilization increased the average HWC content by 0.024 mgC/g dry soil (9.2%) as compared to unfertilized variant. Percentage increases in HWC were greater than for total organic C for all the fertilized variants. Blair *et al.* (2006) have found greater increase of labile C than that of total C in FYM amended variants of long-term experiment. Schulz and Körschens (1998) showed that hot water extractable carbon was more sensitive to different management practices than total C. In our experiment both the total organic C and HWC content in soil was influenced significantly by organic manuring and combined organic and mineral fertilization. HWC content tended to slightly increase in the period of observation (especially in the period of 2002–2006) in all variants (Figure 2) with the fluctuations in individual years.

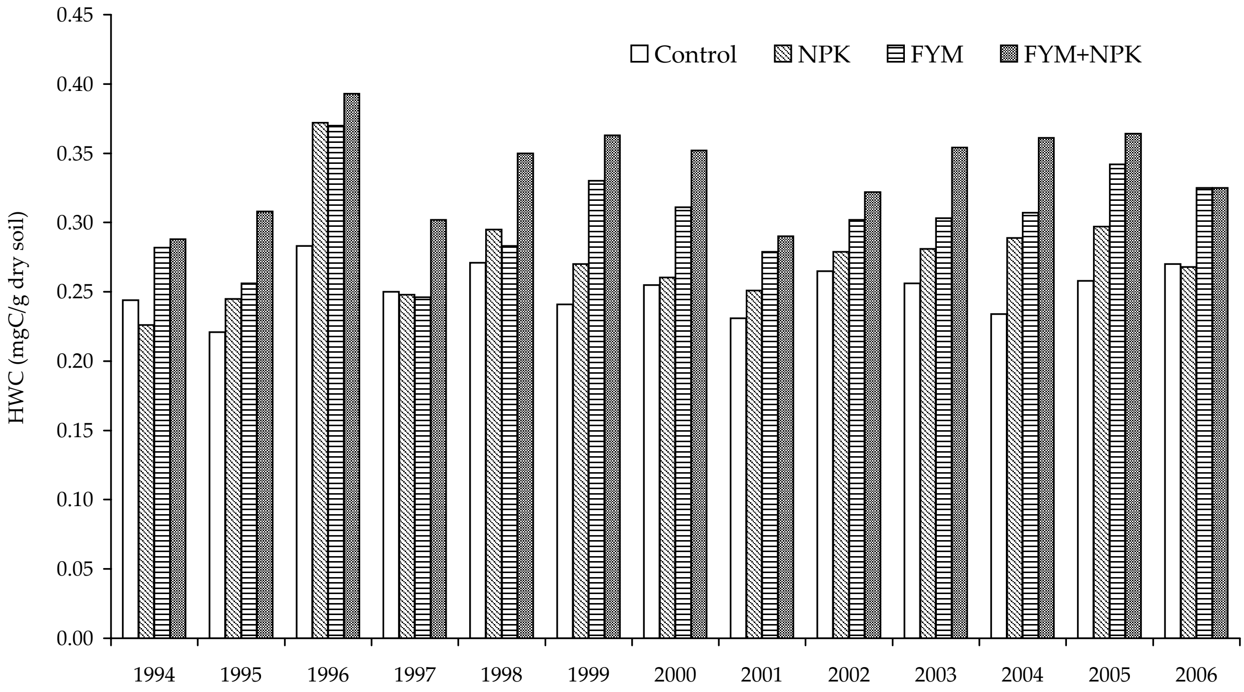

Figure 2. Hot water extractable C (HWC) content in soil samples

Aliphatic compounds

The contents of aliphatic compounds (intensity of C-H signal areas in the 3000–2800 cm^{-1} absorption band region) determined in the bulk soil samples using FTIR spectroscopy are shown in Table 2. The highest content was measured for FYM + NPK followed by FYM and NPK. The aliphatic compounds content in FYM + NPK and FYM variants differed significantly from control unfertlilized variant. The increase of the aliphatic compounds content in NPK variant was not significant compared to control variant. Percentage increases in aliphatic compounds content were higher than for both the total organic C and HWC content. Combined organic and mineral fertilization (FYM + NPK) increased the average aliphatic compounds content by 63.6%, single organic manuring (FYM) increased the average aliphatic compounds content by 48.9% and single NPK fertilization increased the average aliphatic compounds content by 21.0% as compared to unfertilized variant. It was previously shown that the long-term organic manuring increased the aliphatic compounds content in the soil (Šimon 2007b). Ellerbrock *et al.* (1999) showed that the content of CH_2 groups (the band at 2922–2926 cm^{-1}) depended on fertilizer treatment; their content was greatest in soils with cattle manure and PK fertilization. Accumulation of some aliphatic structures in humic substances during the composition of organic matter and a decrease of aliphatic chains following the decomposition of organic matter was observed by Solomon *et al.* (2005). Variation of the aliphatic compounds content in individual variants in the period of observation was greater than for organic C and HWC content and the tendency to slight increase was registered for all variants (Figure 3).

Microbial biomass C

Soil microbial biomass is the living component of soil organic matter and it is involved in nutrient transformation and storage. Carbon contained within the microbial biomass is stored for microbial processes (Rice *et al.* 1996). Microbial biomass is very dynamic and responds to weather, crop input and fertilization. In our experiment, single organic manuring and combined organic and mineral fertilization increased significantly the average content of microbial biomass C in the soil (FYM – amount increase 12.2%, FYM + NPK

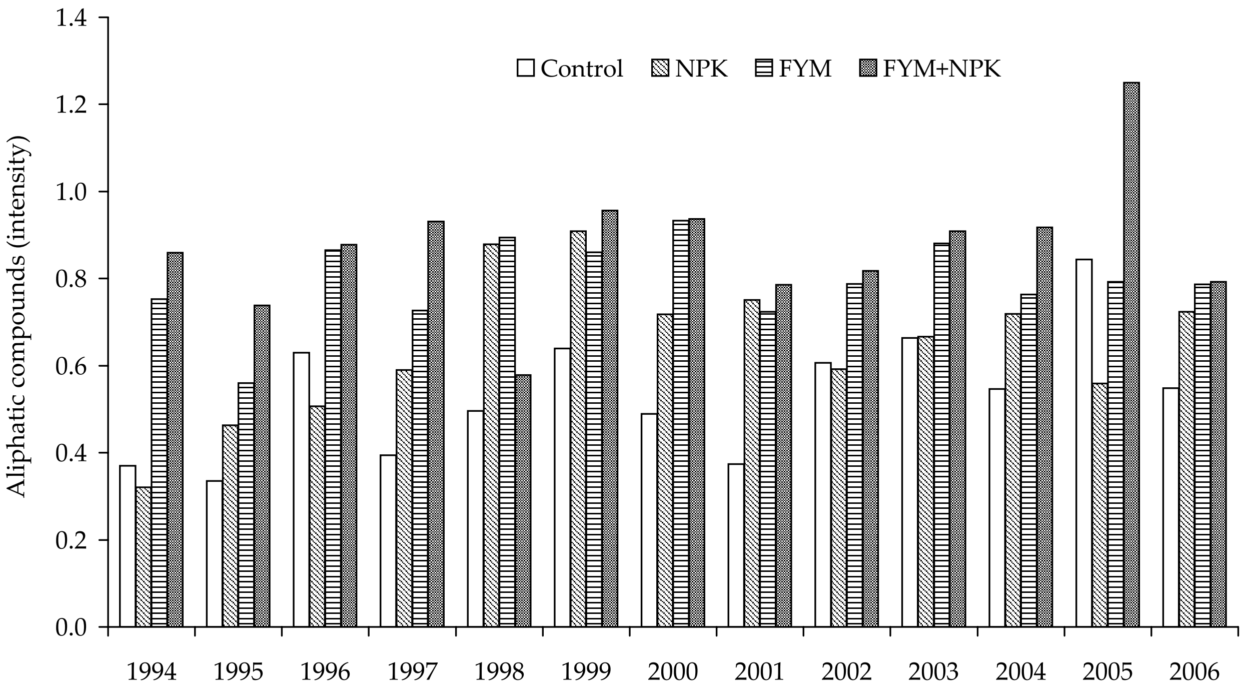

Figure 3. Content of aliphatic compounds (C-H signal area in the 3000–2800 cm^{-1} absorption band region) in soil samples

– 26.9%) (Table 2). On the other hand, NPK application did not increase the content of microbial biomass C compared to control variant. Okano *et al.* (1991) and Ghani *et al.* (2003) even found the negative impact of N fertilizer addition on the microbial biomass in pastoral soils. Seasonal variation of microbial biomass C content in the period of observation was high; the tendency to increase biomass C content was recorded for all variants in the last three years (Figure 4).

Basal respiration activity

Basal respiration activity was measured in the incubation period from 4 to 7 days. This activity is a direct reflection of the degradation (mineralization) of organic C compounds in the soil (Parkin *et al.* 1996). Mineralizable C fluctuates seasonally and its activity and response to treatment may depend on the time of sampling (Haynes 2005). In our experiment average values for basal respiration activity in the period of observation did not differ significantly among the individual variants (Table 2) but seasonal variations were observed (Figure 5). The effect of plants cropped in individual year on the basal respiration was confirmed. After the period of 1995–1996 when lucerne was cropped, basal respiration increased strongly in all variant regardless of treatment and similar situation repeated in the period of 2004–2005. Soon *et al.* (2007) found the increased basal respiration under crop rotation with red clover as compared to the crop rotation without forage legumes. The rate of respiration in organic and mineral fertilized variants has been supported by increased plant residues after the harvest of crops as a source of organic C for microbial decomposition.

Total nitrogen

Periodical input of farmyard manure and NPK fertilizer increased the total nitrogen content in the fertilized variants as compared to control variant (Table 2). Combined organic and mineral manuring (FYM + NPK) increased the average total N content by 18.8%, single organic manuring (FYM) increased the average total N content by 9.1% and single NPK fertilization increased the average total N content by 8.0% as compared to unfertilized variant. The total N content in FYM + NPK variant differed significantly from control variant. The effect of single organic and single

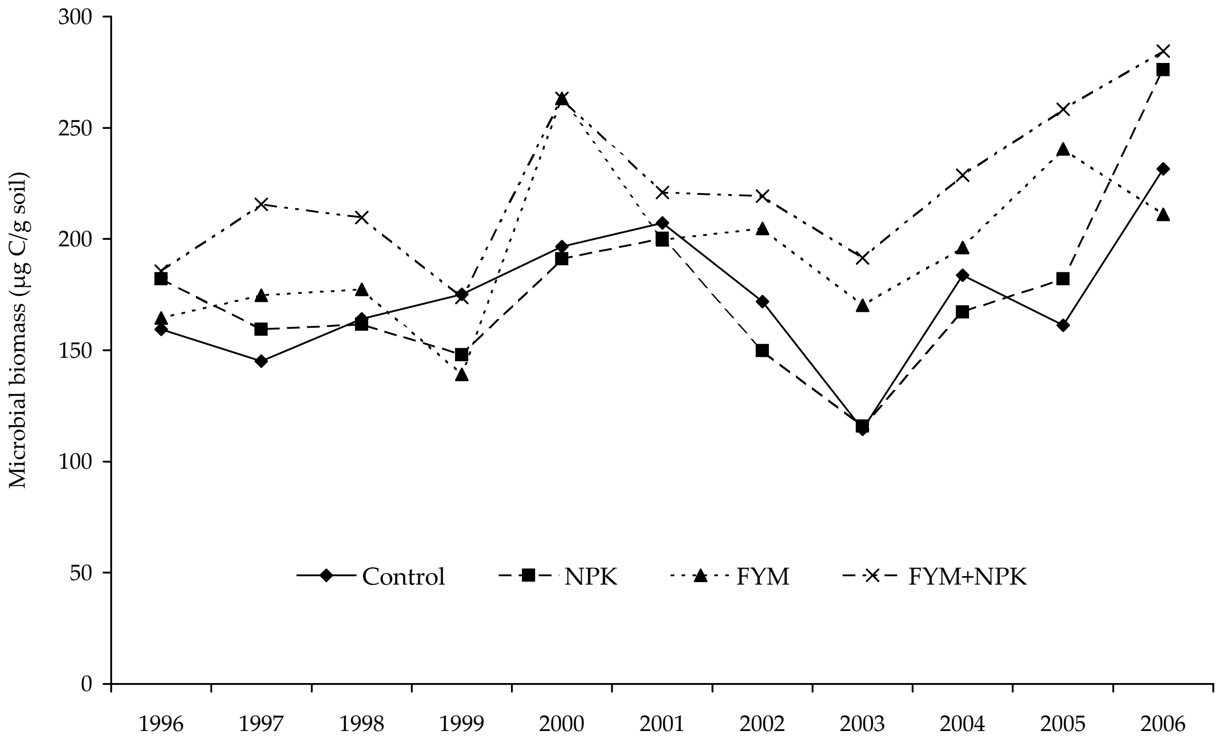

Figure 4. Microbial biomass C in soil samples in the period of 1996–2006

mineral fertilization on the total N content in the soil was very similar even if the average annual N doses applied in FYM variant were only 60% of NPK variant. The part of mineral nitrogen applied to the mineral fertilized variant was lost from soil via several pathways such as denitrification,

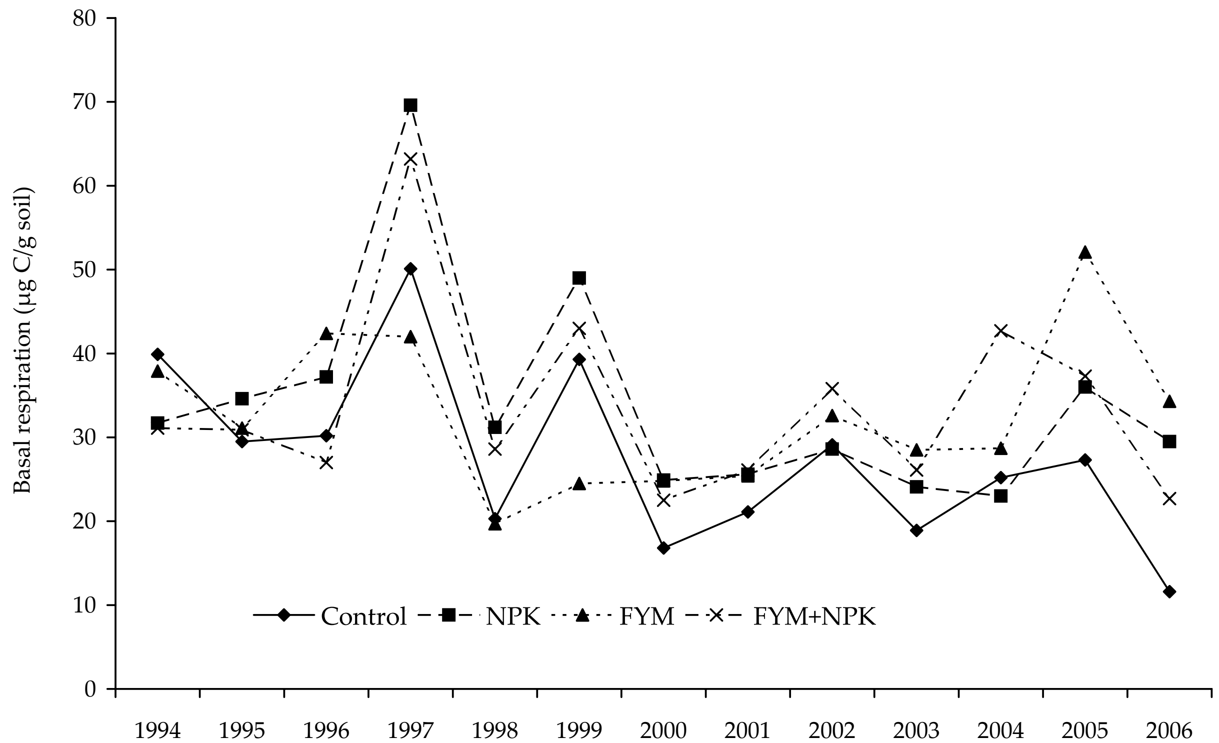

Figure 5. Basal respiration activity in soil samples in the period of 1994–2006

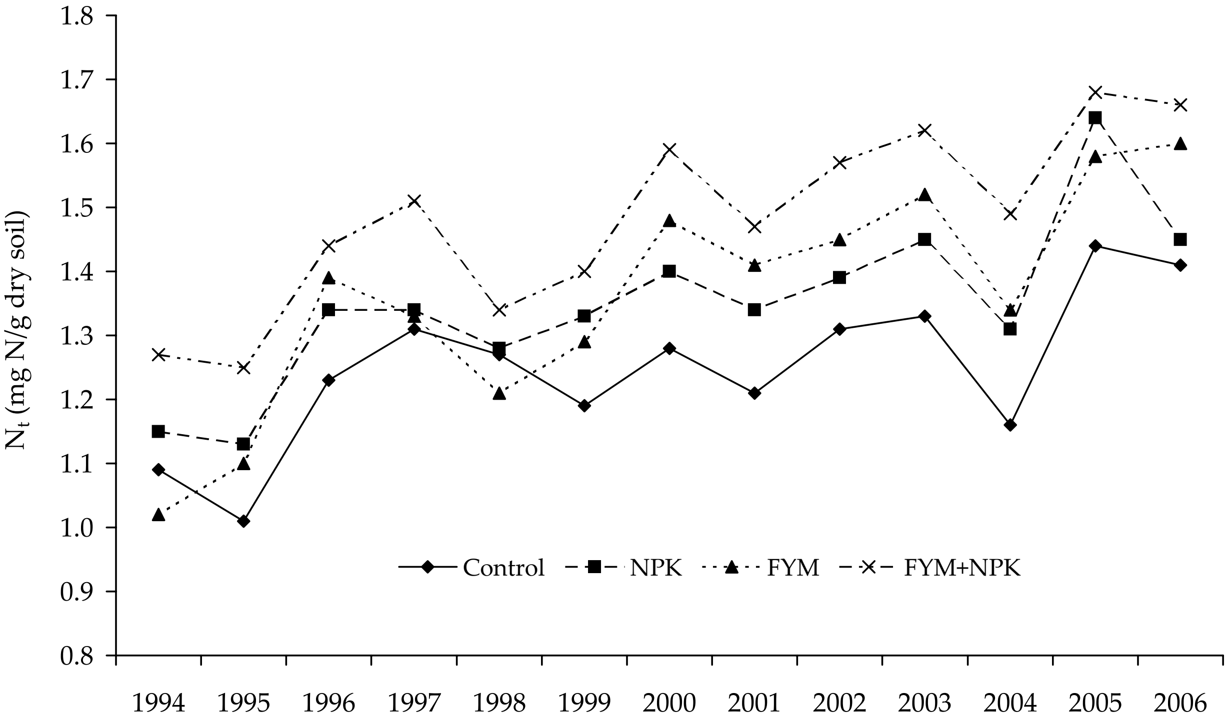

Figure 6. Total nitrogen content (N_t) in soil samples in the period of 1994–2006

volatilization and leaching. On the other hand, higher increase in total nitrogen content in soil periodically manured with FYM + NPK can be attributed to the higher return of organic N from roots and stubble from higher yielding crops. Blair *et al.* (2006) showed that long-term application of 30 t FYM/ha/2 year increased total N by 31% compared to no FYM or mineral fertilizers. Presence of lucerne in crop rotation contributed also positively to the total nitrogen content in soil of all variants due to the symbiotic N_2 fixation. After the period of 1995–1996 when lucerne was cropped, the total N content increased strongly in all variants regardless of treatment. Similar situation repeated in the period of 2004–2005 (Figure 6). Kubát *et al.* (2003) calculated contribution of the crop rotation including 22% lucerne to the nitrogen budget with more than 47 kg N/ha/year.

C:N

The ratio C to N provides information on the capacity of the soil to store and recycle nutrients. The C:N ratio of soils is approximately 10 to 1 and higher ratios may indicate recent additions of manure or plant residues (Sikora & Stott 1996). In our experiment the ratios varied from 9.96 (control) to 10.46 (FYM) and the differences among the variants were not significant (Table 2). The tendency to decline of C:N in all variants was reported in the period of observation due to the nearly constant organic C content but increased total N in this period (Figure 7).

Relationships

All the data collected from each of observed variants were pooled together to examine the correlations among determined parameters. Correlation coefficients (r) at $P < 0.05$ and $P < 0.01$ level of significance are shown in Table 3. Strong significant positive relationships of total organic C and HWC and aliphatic compounds were found, the weak but also significant relationships were determined between total organic C and microbial biomass C and total N. Significant positive relationships of total organic C and aliphatic compounds were received also from the data from other field experiments (Šimon 2007b). HWC was significantly correlated with the aliphatic compounds,

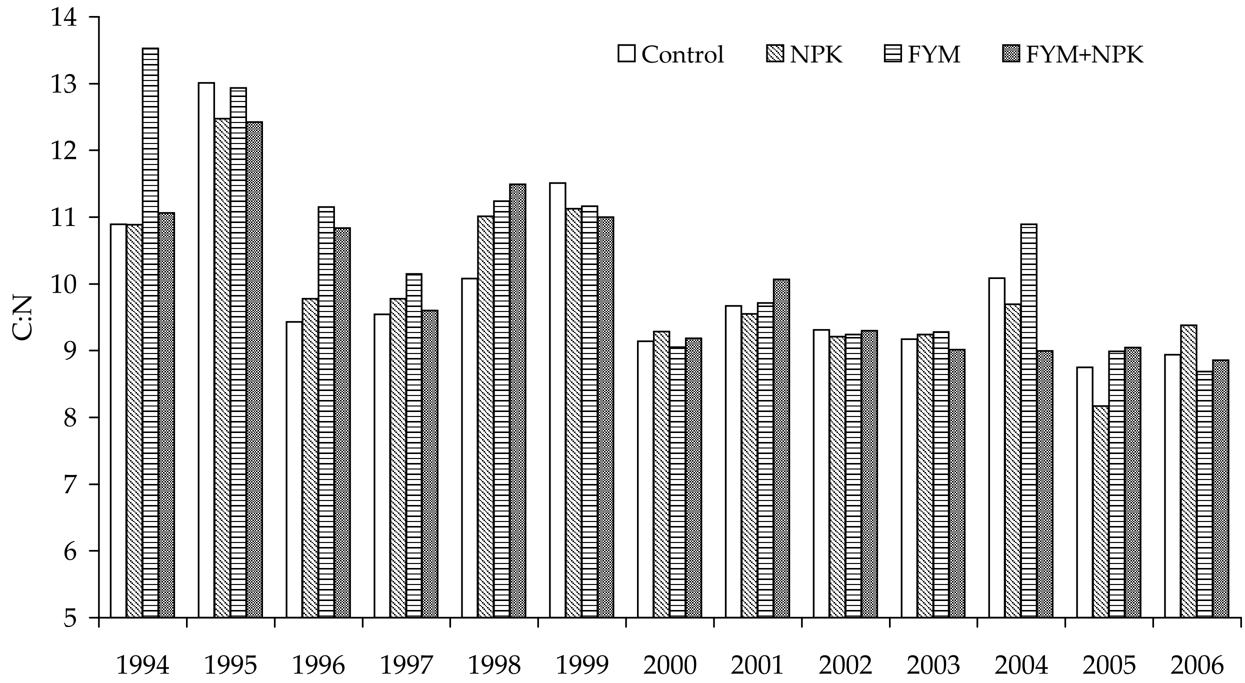

Figure 7. C:N ratio in four variants over the investigated period

and microbial biomass C. Sparkling *et al.* (1998) and Ghani *et al.* (2003) reported strong correlation between microbial biomass C and HWC content in soil. Ghani *et al.* (2003) extracted from soils 3–7 times higher amount of HWC than that extracted as C of microbial biomass. We extracted only 1.5–1.6 times higher amount of HWC from our soil samples. The reason may be that we studied arable soils with substantially lower total organic C content as compared to Ghani *et al.* (2003). In addition, strong positive correlations between HWC, microbial biomass C and aliphatic compounds and total N was found. Basal respiration activity was not correlated with any other parameters determined in soils (Table 3). Respiration activity indicates the intensity of metabolic processes and reflects rather short-term seasonal effects of temperature and humidity changes and the effect of cropped plants and fertilization than long-term changes in carbon metabolism. Respiration activity is therefore an important characteristic of the carbon dynamics in soil (Kubát *et al.* 1999).

Table 3. Relationships between characterised parameters (correlation coefficients)

Parameter	Organic carbon	Hot water extractable carbon	Aliphatic compounds	Microbial biomass C	Basal respiration activity	Total nitrogen
Organic carbon	–	–	–	–	–	–
Hot water extractable carbon	0.682**	–	–	–	–	–
Aliphatic compounds	0.631**	0.648**	–	–	–	–
Microbial biomass C	0.308*	0.381*	0.370*	–	–	–
Basal respiration activity	0.229NS	0.090NS	0.107NS	–0.097NS	–	–
Total nitrogen	0.385*	0.613**	0.603**	0.639**	0.060NS	–

*significant at $P < 0.05$, **significant at $P < 0.01$, NS – non-significant

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