# Aliphatic compounds, organic C and N and microbial biomass and its activity in long-term field experiment

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**ABSTRACT**

The content of aliphatic compounds, hydrophobicity index, organic C and N content and the microbial biomass and respiration activity were analysed in soil samples originating from different plots of a long-term field experiment (variants: nil, NPK – mineral fertilization: 64.6–100 kg/ha/year, FYM – farmyard manure and FYM + NPK) from three blocks (III, IV and B) with different crop rotation. Samples were taken from 0–200 mm layer in 2002 and 2003 (spring and autumn). The plots without any fertilization had the significantly lowest aliphatic compound content compared to variants fertilized by FYM or FYM + NPK in all the evaluated blocks in both years. The variants fertilized only by mineral NPK without any organic fertilization had the slightly increased aliphatic compound content but they did not exceed significantly the control variants in most cases. The aliphatic compound contents correlated significantly with the organic C contents in 2002 and 2003, as well. The values of the hydrophobicity index showed a similar trend like the data mentioned above. Organic manure increased the soil organic nitrogen content, similarly to the carbon content. In variants fertilized by FYM and FYM + NPK the higher microbial biomass content was found comparing to unfertilized variants. Correlations between aliphatic compound content and biomass differed in spring (2002: $r$ = 0.065, 2003: $r$ = 0.068) and autumn (2002: $r$ = 0.407, 2003: $r$ = 0.529). Organically fertilized variants had increased basal respiration, in autumn 2002 the basal respiration was higher in variants fertilized by mineral NPK, too. The highest specific respiration was recorded in the unfertilised plot in block B (autumn 2002 and 2003), where low microbial biomass exhibited high activity. Increased specific respiration was found also in plots fertilized by FYM and FYM + NPK (block III and IV, autumn samplings). Positive significant correlations between microbial biomass content and basal respiration were found in 2002 (spring: $r$ = 0.716) and 2003 (spring: $r$ = 0.765, autumn: $r$ = 0.671).

**Keywords:** long-term field experiment; aliphatic compounds; organic carbon; organic nitrogen; microbial biomass; respiration activity

Agricultural management influences quantitatively the content and composition of soil organic matter. One of the possibilities how to directly and non-destructively study the soil organic matter is infrared spectroscopy, especially the diffuse reflectance technique, commonly known as DRIFT (diffuse reflectance infrared fourier transform spectroscopy) when it is used in conjugation with Fourier transform infrared (FTIR) spectrometer (Capriel et al. 1995). This technique is widely used for qualitative and quantitative analysis of soil organic matter fractions and humic and fulvic acids (Inbar et al. 1990, Wander and Traina 1996, Kalbitz et al. 1999), compost and peat (Niemeyer et al. 1992). Nguyen et al. (1991) used the DRIFT technique to study the range of Australian soils. Madari et al. (1998) used this technique for evaluating the effect of tillage and non-tillage on soil organic matter composition. The DRIFT technique has been also used by Capriel et al. (1995) to determine the hydrophobicity of organic matter of a large number of arable soils widely differing in texture and organic C content. The hydrophobicity of soil organic matter is essentially caused by aliphatic C-H units present in methyl, methylene and methine groups. Capriel et al. (1995) defined hydrophobicity index (HI) as the area of the aliphatic C-H infrared band in the 3000–2800/cm region divided by organic C and they found a close relation between the hydrophobicity of organic matter and texture of soils investigated. The organic matter of sandy soils contained more aliphatic C-H units than the clayey soils. In other words the sandy soils were more hydrophobic. The number of aliphatic C-H units controls the water affinity that influences resistance to microbial degradation, the rate of wetting and adsorption processes. These properties play an essential role in the dynamic of organic matter and the microbial biomass, aggregate stability, water infiltration, leaching of organic and inorganic pollutants, and in the chemical composition and dynamics of dissolved organic matter. In addition to the soil

Supported by the Ministry of Agriculture of the Czech Republic, Project No. MZE-M01-01-01.

texture agricultural management influences the dynamics of the hydrophobic component, too. Capriel (1997) investigated the range of soil samples from long-term experiment field sites with different crop rotation and agricultural management in Germany. The results of his investigations show that agricultural management clearly influences the amount of aliphatic C-H units and implicitly the hydrophobicity of the soil organic matter. A decrease of organic C due to the management is accompanied by a decrease of hydrophobicity as well as of soil microbial activity and aggregate stability. The aim of our study was to evaluate the content of aliphatic compounds that constitute the major part of humic acids, hydrophobicity index, organic C and N content in soil samples of selected blocks of the long-term field experiment with different fertilization and crop rotation. The microbial biomass and basal respiration were determined to complete the data.

## MATERIAL AND METHODS

**Soil samples.** Soil samples were taken from the selected plots of the long-term field experiment in Prague-Ruzyně. Soil type is Orthic Luvisol, clay-loam, developed on dilluvial sediments mixed with loess, soil reaction is neutral ($pH_{KCl}$ is 6.8–7.1) in the whole profile. Four variants (nil – no fertilization, NPK – mineral fertilization, FYM – farmyard manure and FYM + NPK) from three blocks (III, IV and B) have been selected for this study. Crop rotation and organic and mineral N fertilization is shown in Table 1. Crop rotation in blocks III and IV differ just in a one-year shift of the cultivated crops. Soil samples have been taken from 0–200 mm layer in all plots (three partial samples per plot were mixed together) four times (spring, autumn 2002 and 2003). Cultivated crops – block III: sugar beet (2002), spring barley (2003); block IV: spring barley (2002), potatoes (2003) and block B: spring wheat (2002), sugar beet (2003).

**Analysis.** The soil samples were sieved through 2mm sieve, air-dried and ground. For the DRIFT 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 DRIFT spectra were measured on Thermo Nicolet Avatar 320 FTIR spectrometer equipped by Smart Diffuse Reflectance accessory. Three DRIFT spectra (absorption mode, KBr background, 256 scans, data spacing 1.929/cm) were collected for each soil sample. The aliphatic C-H signal area of the samples (3000–2800/cm) was integrated by the spectrometer software (Omnic, version 6a).

Soil microbial biomass C was determined by the fumigation-extraction method according to Vance et al. (1987). Oxidisable carbon ($C_{ox}$) was determined in air dried soil samples by wet combustion according to Alten et al. (1935). Basal respiration $CO_2$-C evolved after 3 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

Table 1. Selected blocks and variants

| | Crop rotation | Variants | Average N doses (kg N/ha/year) |
|---|---|---|---|
| Block III | since 1955<br>9 years:<br>lucerne, lucerne, winter wheat,<br>sugar beet, spring barley, potatoes,<br>winter wheat, sugar beet, spring barley | 114 0 | 0 |
| | | 154 NPK | 64.6 |
| | | 214 FYM | 38.6 |
| | | 254 FYM + NPK | 103.2 |
| Block IV | since 1955<br>9 years:<br>lucerne, lucerne, winter wheat,<br>sugar beet, spring barley, potatoes,<br>winter wheat, sugar beet, spring barley | 114 0 | 0 |
| | | 154 NPK | 64.6 |
| | | 214 FYM | 38.6 |
| | | 254 FYM + NPK | 103.2 |
| Block B | since 1965<br>alternatively sugar beet<br>and spring wheat | 114 0 | 0 |
| | | 184 NPK | 100 |
| | | 214 FYM | 57 |
| | | 284 FYM + NPK | 157 |

NPK – mineral fertilization, FYM – farmyard manure titration with standard HCl. The total nitrogen ($N_t$) was determined in air-dried soil samples on a LECO analyzer.

The data of aliphatic compound contents were processed by analysis of variance followed by the Tukey test, that evaluate the significance of differences among the variants. Correlations for the main soil characteristics were calculated.

## RESULTS AND DISCUSSION

The C-H signal areas in the 3000–2800/cm absorption band region (aliphatic compounds) of the soil samples from three blocks are shown in Tables 2 and 3. The investigated variants of these blocks belong to the same soil type they do not differ in soil texture but they differ in management (crop

Table 2. Aliphatic compounds, $C_{ox}$ and hydrophobicity index of soil samples (2002)

| Variant | Aliphatic compounds | | $C_{ox}$ (g/kg) | | Hydrophobicity index | |
|---|---|---|---|---|---|---|
| | spring | autumn | spring | autumn | spring | autumn |
| III 114 0 | 1.127bc* | 0.906bcd | 11.9 | 12.6 | 0.094 | 0.072 |
| III 154 NPK | 1.243cde | 0.966cde | 13.1 | 13.3 | 0.095 | 0.073 |
| III 214 FYM | 1.513ef | 1.166ef | 13.2 | 14.1 | 0.115 | 0.083 |
| III 254 FYM + NPK | 1.328cde | 1.123ef | 13.8 | 14.6 | 0.096 | 0.077 |
| IV 114 0 | 0.923ab | 0.603a | 12.2 | 12.3 | 0.076 | 0.049 |
| IV 154 NPK | 1.288cde | 0.767abc | 12.8 | 13.0 | 0.101 | 0.059 |
| IV 214 FYM | 1.455def | 0.986de | 13.4 | 14.3 | 0.109 | 0.070 |
| IV 254 FYM + NPK | 1.711f | 1.271f | 14.6 | 14.3 | 0.117 | 0.089 |
| B 114 0 | 0.759a | 0.738ab | 11.7 | 11.9 | 0.065 | 0.062 |
| B 184 NPK | 1.225cd | 0.956cde | 12.3 | 13.2 | 0.100 | 0.072 |
| B 214 FYM | 1.695f | 1.314f | 14.6 | 15.2 | 0.116 | 0.086 |
| B 284 FYM + NPK | 0.768a | 1.287f | 13.8 | 14.7 | 0.056 | 0.088 |

*means within the column followed by the same letter do not differ significantly as determined by Tukey multiple range test ($P < 0.05$)

Table 3. Aliphatic compounds, $C_{ox}$ and hydrophobicity index of soil samples (2003)

| Variant | Aliphatic compounds | | $C_{ox}$ (g/kg) | | Hydrophobicity index | |
|---|---|---|---|---|---|---|
| | spring | autumn | spring | autumn | spring | autumn |
| III 114 0 | 0.523ab | 0.566ab | 11.7 | 12.5 | 0.045 | 0.045 |
| III 154 NPK | 0.721cd | 0.662abc | 12.9 | 12.9 | 0.056 | 0.051 |
| III 214 FYM | 0.818defg | 0.903de | 13.2 | 13.6 | 0.062 | 0.066 |
| III 254 FYM + NPK | 0.921fg | 0.791cd | 13.4 | 13.7 | 0.069 | 0.058 |
| IV 114 0 | 0.663bc | 0.473a | 12.2 | 13.4 | 0.054 | 0.035 |
| IV 154 NPK | 0.667c | 0.640abc | 13.4 | 14.2 | 0.050 | 0.045 |
| IV 214 FYM | 0.880efg | 0.856d | 14.1 | 15.0 | 0.062 | 0.057 |
| IV 254 FYM + NPK | 0.908fg | 1.063ef | 14.6 | 15.8 | 0.062 | 0.067 |
| B 114 0 | 0.501a | 0.747bcd | 11.8 | 12.3 | 0.042 | 0.061 |
| B 184 NPK | 0.788cdef | 0.879de | 12.7 | 13.6 | 0.062 | 0.065 |
| B 214 FYM | 0.956g | 1.167fg | 14.2 | 15.4 | 0.067 | 0.076 |
| B 284 FYM + NPK | 0.742cde | 1.323g | 14.0 | 16.8 | 0.053 | 0.079 |

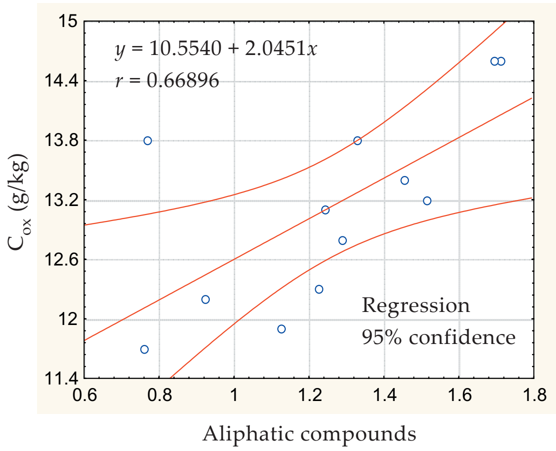


Figure 1. Correlation aliphatic compounds vs. $C_{ox}$ (spring 2002)

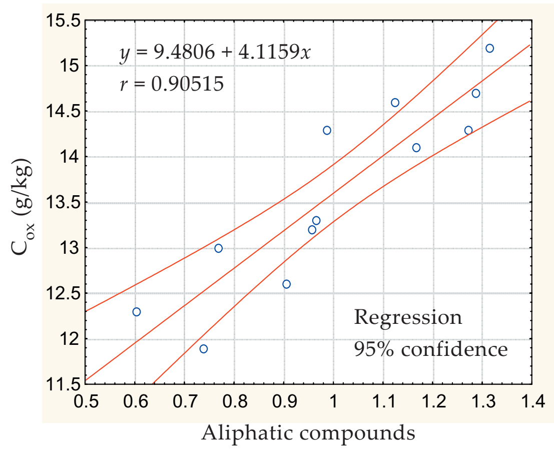


Figure 2. Correlation aliphatic compounds vs. $C_{ox}$ (autumn 2002)

rotation and fertilization, as well). Supposing that the plots used for investigation have the same history of their soil evolution afterwards it is obvious that the differences among the variants in the content of aliphatic C-H units are due to the different management methods used in these plots. Comparing the values of aliphatic compound contents obtained in block III or IV and B that differ in crop rotation it seems that the crop rotation plays in this case a negligible role. Capriel (1997) who evaluated the effect of the management on the soil organic matter found the high differences between different cropping system but he used the long-term field experiment with bare fallow, single crop, crop rotation and grassland. He did not compare the two different crop rotations between themselves. On the other hand when he compared the two plots of the other long-term experiment with the same crop rotation but with fertilization and without fertilization he found the high differences. Similar results have been obtained in our study. The variants without any fertilization had the significantly lowest aliphatic compound content compared to variants fertilized by FYM or FYM + NPK in both years of investigation (Tables 2 and 3). The variants fertilized only by mineral NPK without any organic fertilization had a slightly increased aliphatic compound content but they did not exceed significantly the control variants in most cases. These results confirmed that the decreased or nil organic C input to the soil due to the management of fertilization is accompanied by a decrease of aliphatic compound content that constitutes the major part of humic substances. Simultaneously, higher organic C contents were found in variants fertilized by FYM or FYM + NPK than in unfertilized variants or variants fertilized by mineral fertilization in all blocks (Tables 2 and 3). The aliphatic compound contents highly significantly correlated with the organic C contents ($C_{ox}$) in 2002 and 2003, as well (Figures 1–4). The values of hydrophobicity index showed the similar trend as data mentioned above (Tables 2 and 3). There is more hydrophobic organic matter in variants fertilized by farmyard manure. Organic manure increased the soil organic nitrogen content in 2002

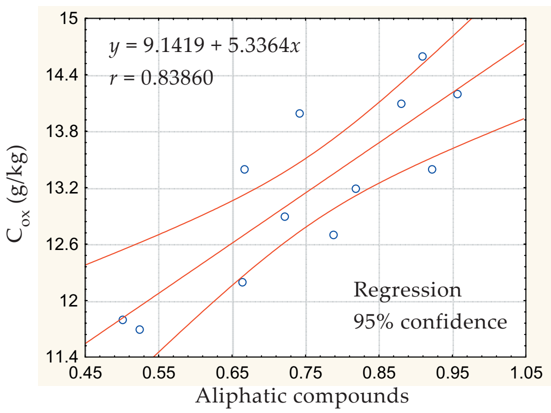


Figure 3. Correlation aliphatic compounds vs. $C_{ox}$ (spring 2003)

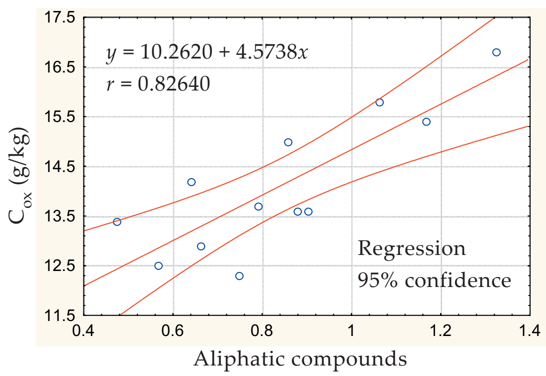


Figure 4. Correlation aliphatic compounds vs. $C_{ox}$ (autumn 2003)

Table 4. Total nitrogen content and C/N in soil samples (2002 and 2003)

| Variant | 2002 | | | | 2003 | | | |
|---|---|---|---|---|---|---|---|---|
| | $N_t$ (g/kg) | | C/N | | $N_t$ (g/kg) | | C/N | |
| | spring | autumn | spring | autumn | spring | autumn | spring | autumn |
| III 114 0 | 1.20 | 1.18 | 9.92 | 10.68 | 1.24 | 1.17 | 9.44 | 10.68 |
| III 154 NPK | 1.38 | 1.25 | 9.50 | 10.64 | 1.40 | 1.30 | 9.21 | 9.92 |
| III 214 FYM | 1.42 | 1.30 | 9.30 | 10.85 | 1.47 | 1.32 | 8.98 | 10.30 |
| III 254 FYM + NPK | 1.45 | 1.42 | 9.52 | 10.28 | 1.52 | 1.37 | 8.82 | 10.00 |
| IV 114 0 | 1.31 | 1.15 | 9.31 | 10.70 | 1.33 | 1.21 | 9.17 | 11.07 |
| IV 154 NPK | 1.39 | 1.26 | 9.21 | 10.32 | 1.45 | 1.34 | 9.24 | 10.60 |
| IV 214 FYM | 1.45 | 1.33 | 9.24 | 10.75 | 1.52 | 1.37 | 9.28 | 10.95 |
| IV 254 FYM + NPK | 1.57 | 1.44 | 9.30 | 9.93 | 1.62 | 1.57 | 9.01 | 10.06 |
| B 114 0 | 1.06 | 1.14 | 11.04 | 10.44 | 1.23 | 1.08 | 9.59 | 11.39 |
| B 184 NPK | 1.26 | 1.17 | 9.76 | 11.28 | 1.31 | 1.23 | 9.69 | 11.06 |
| B 214 FYM | 1.52 | 1.44 | 9.61 | 10.56 | 1.52 | 1.45 | 9.34 | 10.62 |
| B 284 FYM + NPK | 1.58 | 1.46 | 8.73 | 10.07 | 1.54 | 1.66 | 9.09 | 10.12 |

and 2003, similarly to the carbon content (Table 4), and nitrogen was accumulated in soil for a long time in the soil organic matter. 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 and Stott 1996). In our plots the ratios varied from 8.73 to 11.39 and the effect of type of fertilization was not evident probably due to the uncompleted humification processes in FYM manured plots. Soil microbial biomass is the living component of soil organic matter and it is involved in nutrient transformation and storage. In fertilized agricultural systems, microbial biomass can be a significant source and sink of N. 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. The average values of the biomass C content in the investigated plots are shown in Tables 5 and 6. In variants fertilized by FYM and FYM + NPK the higher microbial biomass content was found compared to unfertilised plots especially in spring samplings in 2002 and 2003, as well. Nevertheless, due to the dynamic nature of microbial biomass, the amount of microbial biomass cannot indicate the changes in soil organic matter (increasing, decreasing or equilibrium). Therefore correlations between the biomass and organic C content or aliphatic compound content can differ in spring (2002: $r = 0.065$, 2003: $r = 0.068$) comparing to autumn (2002: $r = 0.407$, 2003: $r = 0.529$). All the calculated correlations were non-significant ($P < 0.05$). The respiration is the basic activity that represents the activity of soil biotic component including microbial activity, invertebrate activity and plant root activity. This activity is a direct reflection of the degradation of organic C compounds in the soil (Parkin et al. 1996). In our study we measured basal respiration that corresponds to microbial activity. The average values of respiration are shown in Tables 5 and 6. Organically fertilized variants had increased basal respiration, similarly to the aliphatic compounds and organic carbon content in both years of investigation. In autumn 2002 the basal respiration was higher also in variants fertilized by mineral NPK (block III and IV), the rate of respiration in these variants has been supported by plant residues after the harvest of crops as a source of organic C for microbial decomposition. The rate of respiration from soil can be related to microbial biomass as an indicator of microbial activity (Rice et al. 1996). Such specific respiration is shown in Table 5 and 6. In our study the highest specific respiration was recorded in unfertilized plots in block B (autumn samplings 2002, 2003) where low microbial biomass exhibited high activity. Increased specific respiration was found also in plots fertilized by FYM and FYM + NPK (block III and IV, autumn samplings). Positive significant correlations between microbial biomass content and basal respiration were found in 2002 (spring: $r = 0.716$) and 2003 (spring: $r = 0.765$, autumn: $r = 0.671$).

Table 5. Microbial biomass, basal and specific respiration in soil samples (2002)

| Variant | Microbial biomass (µg C/g) | | Basal respiration (µg C/g) | | Specific respiration (µg C/µC biomass) | |
|---|---|---|---|---|---|---|
| | spring | autumn | spring | autumn | spring | autumn |
| III 114 0 | 137.44 | 151.22 | 29.9 | 38.3 | 0.22 | 0.25 |
| III 154 NPK | 190.79 | 141.07 | 26.7 | 53.0 | 0.14 | 0.38 |
| III 214 FYM | 183.11 | 114.51 | 37.2 | 50.3 | 0.20 | 0.44 |
| III 254 FYM + NPK | 170.16 | 115.14 | 34.8 | 58.2 | 0.20 | 0.51 |
| IV 114 0 | 171.83 | 105.58 | 27.6 | 39.6 | 0.16 | 0.38 |
| IV 154 NPK | 149.89 | 119.22 | 27.4 | 34.8 | 0.18 | 0.29 |
| IV 214 FYM | 204.80 | 98.01 | 30.3 | 31.8 | 0.15 | 0.32 |
| IV 254 FYM + NPK | 219.35 | 116.30 | 48.8 | 41.0 | 0.22 | 0.42 |
| B 114 0 | 188.14 | 78.15 | 75.8 | 84.2 | 0.40 | 1.08 |
| B 184 NPK | 188.50 | 81.30 | 56.0 | 70.9 | 0.30 | 0.87 |
| B 214 FYM | 247.66 | 118.89 | 84.9 | 95.0 | 0.34 | 0.80 |
| B 284 FYM + NPK | 276.17 | 178.89 | 73.5 | 85.7 | 0.27 | 0.48 |

Table 6. Microbial biomass, basal and specific respiration in soil samples (2003)

| Variant | Microbial biomass (µg C/g) | | Basal respiration (µg C/g) | | Specific respiration (µg C/µC biomass) | |
|---|---|---|---|---|---|---|
| | spring | autumn | spring | autumn | spring | autumn |
| III 114 0 | 91.23 | 116.95 | 14.9 | 13.4 | 0.16 | 0.11 |
| III 154 NPK | 85.84 | 133.23 | 29.1 | 15.0 | 0.34 | 0.11 |
| III 214 FYM | 124.28 | 135.85 | 20.5 | 18.8 | 0.16 | 0.14 |
| III 254 FYM + NPK | 126.42 | 121.21 | 22.6 | 16.0 | 0.18 | 0.13 |
| IV 114 0 | 114.37 | 221.28 | 24.9 | 40.6 | 0.22 | 0.18 |
| IV 154 NPK | 115.95 | 239.81 | 26.0 | 32.9 | 0.22 | 0.14 |
| IV 214 FYM | 170.16 | 227.35 | 29.2 | 44.8 | 0.17 | 0.20 |
| IV 254 FYM + NPK | 191.40 | 284.30 | 27.6 | 53.0 | 0.14 | 0.19 |
| B 114 0 | 283.02 | 145.07 | 74.9 | 68.3 | 0.26 | 0.47 |
| B 184 NPK | 191.34 | 200.99 | 57.9 | 44.1 | 0.30 | 0.22 |
| B 214 FYM | 217.72 | 214.60 | 71.3 | 70.1 | 0.33 | 0.33 |
| B 284 FYM + NPK | 142.50 | 287.10 | 66.2 | 79.9 | 0.46 | 0.28 |

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Received on February 11, 2004

**ABSTRAKT**

**Alifatické sloučeniny, organický C a N a mikrobiální biomasa a její aktivita v dlouhodobém polním pokusu**

Ve vybraných půdních vzorcích z dlouhodobého polního pokusu (varianty: bez hnojení, NPK, hnůj, hnůj + NPK, bloky III, IV a B s různým osevním postupem) byl sledován obsah alifatických sloučenin, index hydrofobicity a obsah organického C a N. Současně byl stanoven obsah mikrobiální biomasy a respirační aktivita. Vzorky byly odebírány z vrstvy 0–200 mm na jaře a na podzim v roce 2002 a 2003. Nehnojené varianty vykazovaly významně nižší obsah alifatických sloučenin ve srovnání s variantami hnojenými hnojem u všech hodnocených bloků v obou letech. Varianty hnojené minerálním NPK měly mírně zvýšený obsah alifatických sloučenin, ale většinou nepřesáhly statisticky významně kontrolní varianty. Obsah alifatických sloučenin vysoce významně koreloval s obsahem organického C v obou letech 2002 i 2003. U indexu hydrofobicity byl pozorován podobný trend jako u obsahu alifatických sloučenin. Organické hnojení zvýšilo obsah organického N a C. U variant hnojených hnojem a hnojem + NPK byl stanoven vyšší obsah mikrobiální biomasy. Korelace mezi obsahem alifatických sloučenin a biomasou byly odlišné na jaře (2002: $r$ = 0,065, 2003: $r$ = 0,068) a na podzim (2002: $r$ = 0,407, 2003: $r$ = 0,529). Organicky hnojené varianty měly zvýšenou bazální respiraci, zvýšená bazální respirace byla zaznamenána též na podzim 2002 u variant hnojených NPK jako následek zapravení rostlinných zbytků po sklizni plodin. Nejvyšší specifická respirace byla stanovena na podzim 2002 a 2003 u nehnojené varianty bloku B, kde nízký obsah mikrobiální biomasy vykázal vysokou aktivitu. Zvýšená specifická respirace byla zjištěna též u variant hnůj a hnůj + NPK (blok III a IV, podzimní odběry). Pozitivní významné korelace mezi obsahem mikrobiální biomasy a bazální respirací byly zjištěny v roce 2002 na jaře ($r$ = 0,716) a v roce 2003 v obou termínech odběru (jaro: $r$ = 0,765, podzim: $r$ = 0,671).

**Klíčová slova:** dlouhodobý polní pokus; alifatické sloučeniny; organický uhlík; organický dusík; mikrobiální biomasa; respirační aktivita

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