Microbial biomass-C determined using $CaCl_2$ and K_2SO_4 as extraction reagents

L. Růžek[1], M. Nováková[1], K. Voříšek[1], I. Skořepová[2], L. Vortelová[1], Z. Kalfařová[1], J. Černý[1], T. Částka[1], W. Barabasz[3]

[1]*Czech University of Agriculture in Prague, Czech Republic*
[2]*Czech Ecological Institute in Prague, Czech Republic*
[3]*University of Agriculture in Krakow, Poland*

ABSTRACT

Microbial biomass-C [MBC] was determined by re-hydration [RHD] technique using two very similar salt solutions in dissociation potency (0.5 mol/l K_2SO_4 [MBC-K] and 0.01 mol/l $CaCl_2$ [MBC-Ca]) in forest, grassland, arable Cambisols [Inceptisols] and Podzols [Spodosols]. MBC-Ca ranged from 254 to 5076 mg/kg dry soil (1.2–4.0% of C_{org}). 114 soil samples were examined in the years 2002 and 2003. Organic C compounds extracted by 0.5 mol/l K_2SO_4 [EC-K] and 0.01 mol/l $CaCl_2$ [EC-Ca] increased in sequence: (1) arable Cambisols (100%), (2) cut and grazed grasslands (547%), (3) forest mineral horizon A_H: 0–50 mm (783%) and (4) Norway spruce forest floor (2421%). The ratio EC-Ca/EC-K reached on average 62% and ranged from 48% to 74%. Correlation between EC-K and EC-Ca values is connected with soil organic matter status; the correlation was very close for Cambisols (r^2 = 0.925), a medium correlation was found for forest floor (r^2 = 0.380) and a weak correlation was observed for Podzols (r^2 = 0.042). The correlation between MBC-K and MBC-Ca was very close in all cases: Cambisols (r^2 = 0.811), Podzols (r^2 = 0.904) and forest floor (r^2 = 0.496). The ratio between organic carbon and organic nitrogen in 0.01 mol/l $CaCl_2$ extracts [EC-Ca/N_{org}] could be declared as a new indicator for soil microbial association status.

Keywords: microbial biomass; K_2SO_4 and $CaCl_2$ extractable C; arable, forest, grassland soils; Cambisols; Inceptisols; Podzols; Spodosols; extraction methods

Soil is a heterogeneous, discontinuous and structured environment (Nannipieri et al. 1990) dominated by a solid phase and wherein microbial life exists in discrete microhabitats, the chemical, physical and biological characteristics of which differ in both time and space. Filip (2001) in his review paper stressed following, ecologically important soil characteristics: microbial biomass, composition of micro-flora (ratio bacteria/fungi, micro-flora of the C-cycle and N-cycle), mineralization processes (CO_2 and NH_4^+ release) and synthesising processes. Hofman et al. (2003) preferred three well-known parameters (microbial biomass carbon [MBC], basal respiration [BR], metabolic coefficient [qCO_2 = BR/MBC]) and five rare parameters (potential respiration [PR], ratio of potential and basal respiration [PR/BR], biomass specific potential respiration [PR/MBC], available organic carbon [EC] and biomass specific available organic carbon [EC/MBC]). Malý et al. (2002) proposed six parameters; three of them were based on microbial biomass nitrogen [MBN]: MBC/C_{org}, MBN/N_{org}, qCO_2 = BR/MBC, qN = N-mineralized/MBN, PR/BR and MBC/MBN. Růžek et al. (2004) evaluated Cambisols [Inceptisols] and Luvisols [Alfisols] by six biological criteria (MBC and five ratios: MBC/C_{org}; EC/MBC; PR/BR; potential/control ammonification [PA/CA]; potential/control nitrification [PN/CN]). Števlíková et al. (2003) evaluated two land managements on stagno-gleic Luvisol without farmyard manure using MBC, MBC/C_{org}, biologically releasable nitrogen [CA + CN] and intensity of nitrification [CN] after and before incubation. Wojewoda and Russel (2003) tested the impact of shelter-belts on soil properties and microbial activity through five criteria: MBC, BR, MBC/C_{org}, dehydrogenase activity, PA/CA.

Supported by the Ministry of Education, Youth and Sports of the Czech Republic, Project No. MSM 412100004 and MSM 6046070901, by the Ministry of Environment of the Czech Republic, Project No. VaV 0101, and by the Ministry of Agriculture of the Czech Republic, Projects No. QC 1254 and QD 1329.

Yakovchenko and Sikora (1998) analyzed microbial biomass C [MBC] using fumigation extraction [FE] and re-hydration [RHD] techniques. Their results for MBC content were in the range 110–440 mg C/kg dry soil for FE and 150–570 mg C/kg dry soil for RHD method. Bauhus (1996) used the determination by FE technique in beech forest floor material (O_F and O_H layers; 2000–3500 mg C/kg), respective 100–520 mg C/kg in mineral horizon A_H (0–50 mm). Růžek et al. (2003, 2004) presented at arable, grassed Luvisols [Alfisols] for RHD method 398–503 mg C/kg dry soil and 396–625 mg C/kg dry soil for arable and grassed Cambisols [Inceptisols] respective 52–1544 mg C/kg dry soil at amended, grassed Anthrosols. Števlíková et al. (2003) documented for RHD method 196–266 mg C/kg dry soil at stagno-gleic Luvisols.

0.5 mol/l K_2SO_4 extracts are widely used in FE and RHD techniques (Vance et al. 1987, Badalucco et al. 1992, Yakovchenko and Sikora 1998 etc.). In the meantime Bauhus (1996) described rare $CaCl_2$ extractable carbon in acid material from beech forest floor (O_F and O_H layers; pH [$CaCl_2$] 3.3, respective 3.0) and from mineral horizon A_H (0–50 mm; pH [$CaCl_2$] 2.9). Similarly, Chander and Joergensen (2002) used $CaCl_2$ extracts in the study of Pb – contaminated soils.

The main aim of this article was to compare two very similar salt solutions in dissociation potency (0.5 mol/l K_2SO_4 and 0.01 mol/l $CaCl_2$) for microbial biomass-C determination in forest, grassland and arable soils.

MATERIAL AND METHODS

Soil samples (basic characteristics are presented in Table 1) from arable, grassland and forest sites were collected in the years 2002 and 2003 in the Central Bohemia region (Lešany, Strážovna, Netvořice, Nedvězí, Všetice, Příbram-Podlesí, Občov) and the North Bohemia region (Kořenov, Václavíkova Studánka) twice a year. Soil samples from the profile (0–150 mm) of arable and grassland soil, from the Norway spruce forest floor (with beech, birch, larch and mountain ash admixture), both O_F and O_H layers, and mineral horizon A_H (0–50 mm) were collected using sampler *Eijkelkamp agrisearch equipment*. They were transported in the cooling box (temperature 6–12°C), adjusted, sieved (mesh 2 mm) and stored in refrigerator (4–6°C). The samples were pre-incubated in the room temperature (22 ± 2°C) 24 hours prior to analyses. In all cases, soil samples with original moisture were used.

The list of the tests used for soil samples characterisation:

– texture: sand, silt, clay content (ISO 112 77) by pipette method
– pH (H_2O), pH (0.2 mol/l KCl), pH (0.01 mol/l $CaCl_2$); 25 ml of reagent and 10 g of air-dried soil sample was shaken (15 minutes) and pH was determined after (over night) sedimentation
– total nitrogen (N_t) – Kjeldahl method
– 0.01 mol/l $CaCl_2$ extractable organic nitrogen (N_{org}) – calculated as the difference between extractable nitrogen (EN-Ca) and the sum of $N\text{-}NO_3^-$ and $N\text{-}NH_4^+$ (Houba et al. 2000, Černý et al. 2003)
– organic carbon (C_{org}; Sims and Haby 1971) – modified colorimetric determination in 600 nm after (over night) sedimentation; 0.5–1 g of air-dried soil sample, respective 0.1 g of forest floor material was shaken with 5 ml of 0.33 mol/l $K_2Cr_2O_7$, followed by injection of 5 ml conc. H_2SO_4 and a digestion (25 minutes) in forced-air ventilation oven at 125°C
– 0.5 mol/l K_2SO_4 extractable organic carbon (EC-K; Vance et al. 1987, Badalucco et al. 1992 etc.) with colorimetric determination in 590 nm; moist soil samples (4–10 g dry wt respective 1–3 g dry wt of forest floor material) were pre-incubated (over night), shaken (30 minutes) with 15–30 ml of reagent at the room temperature (22 ± 2°C); then followed sedimentation (over night), centrifugation (2 ml, 3 minutes; 10 000 rev/minute) and digestion (1:1; 140°C; 20 minutes) at the mixture of (400 mg $K_2Cr_2O_7$, 50 ml conc. H_2SO_4, 20 ml conc. H_3PO_4, 10 ml distilled H_2O).
– 0.01 mol/l $CaCl_2$ extractable organic carbon (EC-Ca; Houba et al. 2000) with colorimetric determination in 590 nm by the same procedure as EC-K
– microbial biomass carbon (MBC) – re-hydration [RHD] technique on the base K_2SO_4 extracts (MBC-K) and $CaCl_2$ extracts (MBC-Ca) after drying at forced-air ventilation oven (65°C, 24 hours; k_C = 0.25; Blagodatskiy et al. 1987)

The following six ratios were calculated:

$(MBC\text{-}K/C_{org}) \times 100$, $(MBC\text{-}Ca/C_{org}) \times 100$,

$(EC\text{-}K/MBC\text{-}K) \times 100$, $(EC\text{-}Ca/MBC\text{-}Ca) \times 100$,

$(EC\text{-}Ca/EC\text{-}K) \times 100$, $EC\text{-}Ca/N_{org}$.

Results were statistically evaluated using analyses of variance (multiple range test) including Fisher's *LSD* method.

RESULTS AND DISCUSSION

Organic carbon extracted by 0.5 mol/l K_2SO_4 (EC-K) and 0.01 mol/l $CaCl_2$ (EC-Ca)

EC-K is a well-known parameter assessing a metabolic status of soil microbial associations. An accumulation of EC-K is a sign of a lower

Table 1. Basic soil parameters

		C_{org} (%)	N_t (%)	pH(H_2O)	pH($CaCl_2$)	Sand (%)[7] 0.063–2 mm	Silt (%)[7] 0.002–0.062 mm	Clay (%)[7] < 0.002 mm
Arable soils[3] 0–150 mm	Cambisols[1]	1.20 ± 0.18[6]	0.14 ± 0.03	6.5 ± 0.4	5.8 ± 0.5	34 ± 13	51 ± 12	15 ± 2
Grassland[4] 0–150 mm	Cambisols	1.92 ± 0.37	0.13 ± 0.02	6.3 ± 0.8	5.2 ± 0.7	48 ± 11	36 ± 12	16 ± 1
	Podzols[2]	5.68 ± 0.30	0.51 ± 0.03	5.6 ± 0.7	4.6 ± 0.7	34 ± 1	57 ± 1	9 ± 1
Forest mineral A_H horizon 0–50 mm	Cambisols	4.57 ± 1.51	0.22 ± 0.07	3.6 ± 0.3	2.9 ± 0.2	51 ± 11	31 ± 7	18 ± 4
	Podzols	6.43 ± 0.33	0.70 ± 0.09	3.8 ± 0.4	3.2 ± 0.4	nd[8]	nd	nd
Forest floor O_F and O_H layers after O_L removal[5]		24.91 ± 4.42	1.22 ± 0.23	3.8 ± 0.3	3.0 ± 0.4	nd	nd	nd
LSD[9] $d_{\alpha\ min}$ 0.05 (0.01)		1.69 (2.23)	0.15 (0.19)	0.4 (0.6)	0.5 (0.6)			

[1]Inceptisols (USDA classification); [2]Spodosols (USDA classification); [3]wheat: winter (+ spring) 25 (+ 3) %, winter rape 25%, corn 17%, barley: winter (+ spring) 11 (+ 11) %, spring poppy 8%; [4]one cut/y respective grazed; [5]Norway spruce (*Picea abies*) culture with beech (*Fagus* sp.), birch (*Betula* sp.), mountain ash (*Sorbus aucuparia* L.) and larch (*Larix decidua* Mill.) admixture; [6]mean ± standard deviation; [7]ISO 11277; [8]not determined; [9]Fisher's least significant difference

stability of soil carbon connected with stress and lyses of microbial population. The usual level (Růžek et al. 2004) in arable and grassed Luvisols [Alfisols] is 40 mg/kg dry soil and 48 mg/kg dry soil in Cambisols [Inceptisols]. As shown in Table 2, EC-K ranged on Cambisols (altitude 370–540 m) and Podzols (altitude 730–900 m) from 31.2 to 216.7 mg/kg dry soil. EC-K increased in the following sequence: [1] arable soil (31 mg/kg dry soil; 0.26% C_{org}), [2] cut and grazed grassland (95–187 mg/kg dry soil; 0.33–0.50% C_{org}) and [3] forest mineral horizon A_H, 0–50 mm (195–217 mg/kg dry soil; 0.33–0.49% C_{org}). The highest level EC-K was measured in Norway spruce forest floor, O_F and O_H layers (597 mg/kg dry soil; 0.22% C_{org}). Badalucco et al. (1992) presented very similar values of EC-K (73–127 mg/kg dry soil) in the profile 0–100 mm at agricultural sites and 306–360 mg/kg dry soil on pinewood forest sites. O'Brien et al. (2003) declared on pinewood forest sites that microbial C was less than 4% of total C, and water – soluble C and K_2SO_4 – extractable C less than 1%. Our results (Table 3), that concern the Norway spruce forest floor, O_F and O_H layers, and forest mineral horizon A_H (0–50 mm) on Cambisols and Podzols, confirmed this conclusion. Organic C, K_2SO_4 – extractable C and microbial biomass C increased in permanent pasture (Haynes et al. 2003) in comparison with undisturbed native grassland. In our experiments, this was typical for cut grassland on Cambisols (75 mg/kg dry soil; SD 28) and for grazed grassland on the same soil type (114 mg/kg dry soil; SD 24); the difference (52%) was non-significant.

Determination of soil organic carbon in the extracts by 0.01 mol/l $CaCl_2$ (EC-Ca) is yet rarely used as a parameter indicating soil biological quality. Chander and Joergensen (2002) referred a mutual relation between $CaCl_2$ – extractable C, microbial biomass C, ergosterol content and CO_2 production. Bauhus (1996) also used $CaCl_2$ – extractable C during study of C and N mineralization in an acid forest soils in Lower Saxony. In our study, we determined that 10 mg EC-Ca /kg dry soil (SD 5) corresponded to 52% of EC-K (0.08% of C_{org}) on arable Cambisols [Inceptisols]. In the case of grassland Cambisols and Podzols it was 57 (SD 21) mg EC-Ca/kg dry soil (64% EC-K; 0.31% of C_{org}),whereas in the case of grassland Podzols it was 112 (SD 27) mg EC-Ca/kg dry soil (64% EC-K; 0.20% of C_{org}),Norway spruce forest floor (O_F and O_H layers) had 444 (SD 211) mg/kg dry soil (74% EC-K; 0.16% of C_{org}). In the A_H horizon (0–50 mm) was found 143 (SD 46) for Cambisols (73% EC-K; 0.38% of C_{org}) and 89 (SD 30) mg EC-Ca/kg dry soil for Podzols (41% EC-K; 0.14% of C_{org}). In all cases,

Table 2. Organic carbon and nitrogen extracted by 0.5 mol/l K_2SO_4 and 0.01 mol/l $CaCl_2$ respectively

		EC-K[7]	EC-Ca[8]	EC-Ca/EC-K (%)	N-NH_4^{+9}	N-NO_3^{-10}	N_{org}[11]	EC-Ca/N_{org}
Arable soils[3] 0–150 mm	Cambisols[1]	31.2 ± 11.4[6] n = 66	9.9 ± 4.8 n = 18	52	3.7 ± 1.9	8.2 ± 6.6	1.9 ± 0.2	7
Grassland[4] 0–150 mm n = 16	Cambisols	94.6 ± 31.7	56.9 ± 20.7	67	1.0 ± 0.6	14.0 ± 13.5	1.4 ± 1.5	55
	Podzols[2]	187.0 ± 32.3	111.5 ± 27.2	59	3.0 ± 2.3	9.2 ± 8.3	4.3 ± 1.6	28
Forest mineral A_H horizon 0–50 mm n = 16	Cambisols	195.1 ± 57.3	142.9 ± 46.1	73	12.7 ± 10.7	3.1 ± 2.8	5.2 ± 5.1	42
	Podzols	216.7 ± 100.4	89.3 ± 29.8	48	19.5 ± 13.4	4.0 ± 2.1	5.5 ± 5.9	66
Forest floor O_F and O_H layers after O_L removal[5] n = 16		564.2 ± 147.5	430.9 ± 237.2	74	45.7 ± 49.0	14.3 ± 13.8	28.3 ± 25.5	31
LSD^{12} $d_{\alpha\ min}$ 0.05 (0.01)		63.5 (84.0)	120.7 (160.6)	23 (30)	32.5 (43.4)	11.0 (14.7)	15.6 (20.8)	66 (88)

[1, 2, 3, 4, 5, 6]see Table 1; [7]0.5 mol/l K_2SO_4 extractable organic C (mg/kg dry soil); [8]0.01 mol/l $CaCl_2$ extractable organic C (mg/kg dry soil); [9]0.01 mol/l $CaCl_2$ extractable ammonium N (mg/kg dry soil); [10]0.01 mol/l $CaCl_2$ extractable nitrate N (mg/kg dry soil); [11]0.01 mol/l $CaCl_2$ extractable organic N (mg/kg dry soil); [12]Fisher's least significant difference

Table 3. Microbial biomass C and ratios with soil organic carbon pools

		MBC-K[7]	MBC-Ca[8]	MBC-K/C_{org} (%)	MBC-Ca/C_{org} (%)	EC-K/MBC-K (%)	EC-Ca/MBC-Ca (%)
Arable soils[3] 0–150 mm	Cambisols[1]	407.7 ± 99.6[6] n = 66	254.2 ± 76.9 n = 18	3.4	2.1	8.2	4.2
Grassland[4] 0–150 mm n = 16	Cambisols	956.9 ± 272.5	343.4 ± 156.6	5.3	2.0	11.1	19.7
	Podzols[2]	1823.3 ± 723.1	1051.9 ± 793.3	3.2	1.8	12.1	16.8
Forest mineral A_H horizon 0–50 mm n = 16	Cambisols	1630.2 ± 550.2	1596.2 ± 661.6	3.7	3.6	13.1	10.9
	Podzols	3059.8 ± 809.2	2577.3 ± 1062.4	4.7	4.0	7.8	3.7
Forest floor O_F and O_H layers after O_L removal[5] n = 16		4792.7 ± 1039.3	5076.1 ± 1321.4	2.0	2.1	12.6	9.0
LSD^9 $d_{\alpha\ min}$ 0.05 (0.01)		502.3 (664.4)	835.2 (1110.8)	0.9 (1.2)	0.9 (1.3)	4.7 (6.2)	6.1 (8.1)

[1, 2, 3, 4, 5, 6]see Table 1; [7]microbial biomass C using K_2SO_4 extraction (mg/kg dry soil; re-hydration technique; k_C = 0.25); [8]microbial biomass C using $CaCl_2$ extraction (mg/kg dry soil; re-hydration technique; k_C = 0.25); [9]Fisher's least significant difference

the level of EC-K was higher than EC-Ca, but only two differences were significant ($P < 0.05$).

The correlation between EC-K and EC-Ca values (Tables 4–6) is connected with soil organic matter status. The correlation was very close for Cambisols (r = 0.962; r^2 = 0.925; n = 82), medium correlation was found for Norway spruce forest floor (r = 0.617; r^2 = 0.380; n = 16) and weak correlation character-

ised Podzols (r = 0.205; r^2 = 0.042; n = 16). The level of correlation between EC-K and EC-Ca values is probably given with strong (medium, weak) relations among EC-K and C_{org} or pH / EC-Ca and C_{org} or pH.

Microbial biomass carbon determined by rehydration [RHD] technique using K_2SO_4 and $CaCl_2$ extraction (MBC-K; MBC-Ca)

MBC-K (Table 3) ranged from 408 to 4793 mg/kg dry soil (2.0–4.7% of C_{org}) in different ecosystems (arable soil, cut and grazed grassland, forest mineral horizon A_H and Norway spruce forest floor). The highest values correspond with Bauhus (1996), medium values with Hofman et al. (2004) and the lowest values with Yakovchenko and Sikora (1998). MBC-Ca was very similar and ranged from 254 to 5076 mg/kg dry soil (1.2–4.0% of C_{org}).

Correlations between MBC-K and MBC-Ca were very close in all cases: Cambisols (r = 0.900; r^2 = 0.811; n = 82), Podzols (r = 0.951; r^2 = 0.904; n = 16) and Norway spruce forest floor (r = 0.704; r^2 = 0.496; n = 16). MBC-Ca values increased on Cambisols in the following sequence: [1] arable 254 mg/kg dry

Table 4. Correlation coefficients between EC-K and EC-Ca, respectively, and other characteristics (n = 82) in Cambisols

		EC-Ca[2]	C_{org}	N_t	N_{org}[3]	pH (H_2O)	pH ($CaCl_2$)	MBC-K[4]	MBC-Ca[5]
EC-K[1]	r	0.962***	0.838***	0.459	0.772***	–0.790***	–0.688**	0.808***	0.719***
	r^2	0.925	0.702	0.211	0.596	0.623 neg.	0.473 neg.	0.653	0.517
EC-Ca[2]	r	0.962***	0.773***	0.398	0.636**	–0.863***	–0.762***	0.762***	0.731***
	r^2	0.925	0.597	0.158	0.404	0.745 neg.	0.580 neg.	0.581	0.534

[1]0.5 mol/l K_2SO_4 extractable organic C (mg/kg dry soil); [2]0.01 mol/l $CaCl_2$ extractable organic C (mg/kg dry soil); [3]0.01 mol/l $CaCl_2$ extractable organic N (mg/kg dry soil); [4]microbial biomass C using K_2SO_4 extraction; [5]microbial biomass C using $CaCl_2$ extraction; *** = P < 0.001, ** = P < 0.01, * = P < 0.05

Table 5. Correlation coefficients between EC-K and EC-Ca, respectively, and other characteristics (n = 16) in Podzols

		EC-Ca[2]	C_{org}	N_t	N_{org}[3]	pH (H_2O)	pH ($CaCl_2$)	MBC-K[4]	MBC-Ca[5]
EC-K[1]	r	0.205	0.391	0.453	0.619*	–0.080	–0.037	–0.007	–0.066
	r^2	0.042	0.152	0.205	0.383	0.006 neg.	0.001 neg.	0.000 neg.	0.004 neg.
EC-Ca[2]	r	0.205	–0.358	–0.383	0.381	0.297	0.295	–0.173	–0.126
	r^2	0.042	0.128 neg.	0.147 neg.	0.145	0.088	0.087	0.030 neg.	0.016 neg.

[1, 2, 3, 4, 5]see Table 4; *** = P < 0.001, ** = P < 0.01, * = P < 0.05

Table 6. Correlation coefficients between EC-K and EC-Ca, respectively, and other characteristics (n = 16) in forest floor[6] (O_F and O_H layers)

		EC-Ca[2]	C_{org}	N_t	N_{org}[3]	pH (H_2O)	pH ($CaCl_2$)	MBC-K[4]	MBC-Ca[5]
EC-K[1]	r	0.617*	0.466	0.066	0.080	–0.086	0.361	0.040	0.119
	r^2	0.380	0.217	0.004	0.006	0.007 neg.	0.130	0.002	0.014
EC-Ca[2]	r	0.617*	0.455	–0.139	0.393	–0.213	0.212	–0.011	0.165
	r^2	0.380	0.207	0.019 neg.	0.155	0.045 neg.	0.045	0.000 neg.	0.027

[1, 2, 3, 4, 5]see Table 4; [6]Norway spruce (*Picea abies*) culture with beech (*Fagus* sp.), birch (*Betula* sp.), mountain ash (*Sorbus aucuparia* L.) and larch (*Larix decidua* Mill.) admixture; *** = P < 0.001, ** = P < 0.01, * = P < 0.05

soil (2.1% of C_{org}), [2] grazed grassland 258 mg/kg dry soil (1.2% of C_{org}), [3] cut grassland 429 mg/kg dry soil (2.7% of C_{org}), [4] forest mineral horizon A_H 1596 mg/kg dry soil (3.6% of C_{org}), [5] Norway spruce forest floor 5076 mg/kg dry soil (2.1% of C_{org}). MBC-Ca values on Podzols reached the same sequence.

Soil extraction by 0.01 mol/l $CaCl_2$ is very suitable (Houba et al. 2000) for determination of: pH; acceptable forms of risk elements (Cd, Pb, As, Zn, Cr, Ni and Co); acceptable forms of nutrients (P, K, Mg, Na, Mn, extractable-N, ammonium-N, nitrate-N, N_{org}, C_{org}); microbial biomass-C by FE and RHD technique; microbial biomass-N by FE technique, and many others. Aydinalp and Katkat (2004) declared for 0.01 mol/l $CaCl_2$ a close negative correlation with pH and a close positive correlation with cation exchange capacity [CEC].

Ratios EC-K/MBC-K and EC-Ca/MBC-Ca

These ratios express metabolic status of soil microbial associations. The level lower than 10% is a sign for a good soil microbial population status and it is a result of its active metabolism (Růžek et al. 2004). This level was confirmed on arable Cambisols and in forest mineral A_H horizons on Podzols. A dramatic difference was observed between cut and grazed grassland (EC-K/MBC-K: 6.6% and 15.5%, respectively; EC-Ca/MBC-Ca: 12.2% and 27.2%, respectively). These ratios responded very sensitively to a negative impact of grazing on soil microbial parameters. Ghani et al. (2003) that evaluated the influence of the grazing intensity on the soil biological properties gave a very similar conclusion. They declared a negative impact of sheep/beef and dairy pastures. Hejduk and Hrabě (2003) explained a negative impact of continuous grazing (1.7–2.0 cattle units/hectare) through the mediation of non-significant decrease of dry weight and distribution of underground plant biomass. A lack of dead plant and root residues has an influence on microbial metabolism. The ratios EC-K/MBC-K and EC-Ca/MBC-Ca evaluate very simply and effectively these changes.

Ratios EC-Ca/EC-K and EC-Ca/N_{org}

The ratio (EC-Ca/EC-K) × 100 (Table 2) expresses the difference in the content of organic C-compounds between soil extracts by 0.5 mol/l K_2SO_4 and 0.01 mol/l $CaCl_2$. The ratio ranged from 48% (Podzols; forest mineral horizon A_H; pH-$CaCl_2$ 3.2) to 74% (Norway spruce forest floor; pH-$CaCl_2$ 3.0), average value is 62%. These marginal values were determined in acid forest soils characterised with the same pH level but with very different content of C_{org} (6.43%, respective 24.91%).

Ratio between organic carbon and organic nitrogen in 0.01 mol/l $CaCl_2$ soil extracts EC-Ca/N_{org} (Table 2) could be declared as an indicator for soil microbial association status, which ranged from 7 to 66 in our 114 soil samples. The lowest values we found in arable Cambisols (7), in cut mountain grassland on Podzols (28) and in Norway spruce forest floor (31). The higher values characterized situation in forest mineral horizon A_H on Cambisols (42), cut grassland on Cambisols (52), grazed grassland on Cambisols (58) and in forest mineral horizon A_H on Podzols (66).

Acknowledgements

We are grateful to Dagmar Musilová (Ontario, Canada) for English correction.

REFERENCES

Aydinalp C., Katkat A.V. (2004): The comparison of extraction methods for evaluating some heavy metals in polluted soils. Plant, Soil and Environment, *50*: 212–217.

Badalucco L., Gelsomino A., Dell'Orco S., Greco S., Nannipieri P. (1992): Biochemical characterisation of soil organic compounds extracted by 0.5M K_2SO_4 before and after chloroform fumigation. Soil Biology and Biochemistry, *24*: 569–578.

Bauhus J. (1996): C and N mineralization in an acid forest soil along a gap – stand gradient. Soil Biology and Biochemistry, *28*: 923–932.

Blagodatskiy S.A., Blagodatskaya E.V., Gorbenko A.Y., Panikov N.S. (1987): A re-hydration method of determining the biomass of microorganisms in soil. Soviet Soil Science, *19*: 119–126.

Chander K., Joergensen R.G. (2002): Decomposition of C^{14} labelled glucose in a Pb – contaminated soil remediated with synthetic zeolite and other amendments. Soil Biology and Biochemistry, *34*: 643–649.

Černý J., Balík J., Pavlíková D., Zitková M., Sýkora K. (2003): The influence of organic and mineral nitrogen fertilizers on microbial biomass nitrogen and extractable organic nitrogen in long-term experiments with maize. Plant, Soil and Environment, *49*: 560–564.

Filip Z. (2001): Ecological, legal and methodical approaches to biological indication of soil quality. In: Proceedings of the 50th Anniversary Conference Crop science on the verge of the 21st century – opportunities and challenges, RICP Prague: 136–140.

Ghani A., Dexter M., Perrott K.W. (2003): Hot-water extractable carbon in soils: a sensitive measurement for determining impacts of fertilisation, grazing and cultivation. Soil Biology and Biochemistry, *35*: 1231–1243.

Haynes R.J., Dominy C.S., Graham M.H. (2003): Effect of agricultural land use on soil organic matter status and the composition of earthworm communities in KwaZulu-Natal, South African Agricultural Ecosystem of Environment, *95*: 453–464.

Hejduk S., Hrabě F. (2003): Influence of different systems of grazing, type of swards and fertilizing on underground phytomass of pastures. Plant, Soil and Environment, *49*: 18–23.

Hofman J., Buchlebová J., Dušek L., Doležal L., Holoubek I., Anděl P., Ansorgová A., Malý S. (2003): Novel approach to monitoring of the soil biological quality. Environment International, *28*: 771–778.

Hofman J., Švihálek J., Holoubek I. (2004): Evaluation of functional diversity of soil microbial communities – a case study. Plant, Soil and Environment, *50*: 141–148.

Houba V.J.G., Temminghoff E.J.M., Gaikhorst G.A., van Vark W. (2000): Soil analysis procedure using 0.01M calcium chloride as extraction reagent. Communications in Soil Science and Plant Analyses, *31*: 1299–1396.

ISO 11277 (1998): Soil quality-determination of particle size distribution in mineral soil material. Method by sieving and sedimentation following removal of soluble salt organic matter and carbonates. International Organization for Standardization, Geneve, Switzerland.

Malý S., Hofman J., Dušek L. (2002): Bioindicative value of eco-physiological indices in routine evaluation of soils – a pilot monitoring study in the Czech Republic. Bodenkultur, *53*: 105–114.

Nannipieri P., Greco S., Ceccanti B. (1990): Ecological significance of the biological activity in soil. In: Bollag J.M., Stotzky G. (eds.): Soil Biochemistry, Vol. 6, Marcel Dekker Inc., New York: 293–353.

O'Brien N.D., Attiwill P.M., Weston C.J. (2003): Stability of soil organic matter in *Eucalyptus regnans* forests and *Pinus radiata* plantations in southeastern Australia. Forest Ecology Management, *185*: 249–261.

Růžek L., Voříšek K., Strnadová S., Nováková M., Barabasz W. (2004): Microbial characteristics, carbon and nitrogen content in cambisols and luvisols. Plant, Soil and Environment, *50*: 196–204.

Růžek L., Voříšek K.,Vráblíková J., Strnadová S., Vráblík P. (2003): Chemical and biological characteristics of reclaimed soils in the Most region (Czech Republic). Plant, Soil and Environment, *49*: 346–351.

Sims J.R., Haby V.A. (1971): Simplified colorimetric determination of soil organic matter. Soil Science, *112*: 137–141.

Števlíková T., Vjatráková J., Javoreková S., Mateová S. (2003): Effect of land management without farmyard manure application on the amount and the activity of soil microbial biomass. Plant, Soil and Environment, *49*: 352–358.

Vance E.D., Brookes P.C., Jenkinson D.S. (1987): An extraction method for measuring soil microbial biomass C. Soil Biology and Biochemistry, *19*: 703–707.

Wojewoda D., Russel S. (2003): The impact of a shelter belts on soil properties and microbial activity in an adjacent crop field. Polish Journal of Ecology, *51*: 291–307.

Yakovchenko V.P., Sikora L.J. (1998): Modified dichromate method for determining low concentrations of extractable organic carbon in soil. Communication in Soil Science and Plant Analyses, *29*: 421–433.

Received on September 30, 2004

ABSTRAKT

Uhlík mikrobní biomasy stanovený v extrakčních činidlech $CaCl_2$ a K_2SO_4

Uhlík mikrobní biomasy [MBC] byl stanoven rehydratační [RHD] technikou s využitím dvou disociačně velmi blízkých roztoků solí 0,5 mol/l K_2SO_4 [MBC-K] a 0,01 mol/l $CaCl_2$ [MBC-Ca] v lesních, lučních, orných kambizemích a podzolech. MBC-Ca se pohyboval v pásmu od 254 do 5076 mg/kg sušiny (1,2–4,0 % C_{org}). 114 půdních vzorků bylo analyzováno v letech 2002 a 2003. Byl porovnáván organický uhlík, extrahovatelný 0,5 mol/l K_2SO_4 [EC-K] a 0,01 mol/l $CaCl_2$ [EC-Ca], který narůstal v pořadí: (1) orné půdy (100 %), (2) sečené a spásané louky (547 %), (3) lesní minerální horizont A_H: 0–50 mm (783 %) a (4) lesní organický horizont (2421 %). Poměr (EC-Ca/EC-K) × 100 dosahoval v průměru 62 % v pásmu od 48 do 74 %. Korelace mezi hodnotami EC-K a EC-Ca souvisela se stavem půdní organické hmoty a byla na kambizemích velmi úzká (r^2 = 0,925), v lesním organickém horizontu střední (r^2 = 0,380) a v podzolech slabá (r^2 = 0,042). Korelace mezi uhlíkem mikrobní biomasy [MBC-K a MBC-Ca] byla ve všech případech velmi úzká: u kambizemí (r^2 = 0,811), u podzolů (r^2 = 0,904) i u lesního organického horizontu

(r^2 = 0,496). Poměr mezi organickým uhlíkem a organickým dusíkem v extraktech 0,01 mol/l $CaCl_2$ z vlhké půdy [EC-Ca/N_{org}] může být novým velmi dobrým indikátorem podmínek pro rozvoj půdních mikrobních společenstev.

Klíčová slova: mikrobní biomasa; $CaCl_2$ a K_2SO_4 extrahovatelný C; orné, lesní, luční půdy; kambizemě; podzoly; extrakční metody

Corresponding author:

Doc. Ing. Lubomír Růžek, CSc., Česká zemědělská univerzita v Praze, 165 21 Praha 6-Suchdol, Česká republika
phone: + 420 224 382 567, fax: + 420 224 382 755, e-mail: ruzek@af.czu.cz