Microbiological parameters of soil set aside before and after desiccation

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ABSTRACT

Herbaceous cover of luvic chernozem (seven years set aside) with legumes, perennial grasses and their mixture was desiccated by the glyphosate herbicide Roundup Biaktiv (5 l/ha at July 2003 and 4 l/ha at June 2004). 165 soil samples were collected before (November 2002–July 2003) and after desiccation (September 2003–August 2004). Desiccation had a strong positive effect on immobilization of organic carbon from herbaceous cover and underground biomass to microbial biomass carbon (MBC) and to soil organic carbon (C_{org}), respectively. A significant increase after desiccation ($P < 0.01$) was confirmed in the parameters: C_{org}, MBC and in the ratio MBC/C_{org}. At the same time the desiccation had a retardation effect on mineralization of C_{org}, N_{org} and S_{org}. A significant decrease after desiccation ($P < 0.01$) was confirmed in the basal and potential respiration (with nitrogen, with nitrogen and glucose), actual content of N-NH_4^+ without and with pre-incubation, control ammonification, and ($P < 0.05$) in arylsulphatase activity. The actual content of NO_3^- and the control nitrification increased significantly ($P < 0.01$) after desiccation. Plots management before desiccation included black and spontaneous fallow, mulching (one or two per year) and cutting (three times per year), plots with 4 legumes, 4 grasses and their mixtures. Mulching variants showed the best results both before and after desiccation. The soils with cutting treatment ranked 8^{th}–10^{th} out of 11 studied combinations.

Keywords: land set aside; fallow; desiccation; Roundup Biaktiv; microbial biomass; K_2SO_4 extractable carbon; biological activity; respiration; nitrification; ammonification; arylsulphatase

Soil quality represents an integral value of compositional structures and functions of terrestrial soils in relation to their different use and to long-term environmental conditions on site (Filip 2001). This author stressed in his review paper following ecologically important soil characteristics: microbial biomass, composition of microflora, mineralization and synthesising processes. Some other authors (Dušek et al. 1997, Škoda et al. 1997, Růžek et al. 2001, 2003, 2004, 2005) preferred precise descriptions using biological characteristics like respiration, ammonification, nitrification, enzyme activities and microbial biomass carbon determination.

Many methods determining both quantity of microorganisms and their activity can be used for the characterisation of soil biological quality. Voříšek et al. (2002) used three or eight parameters (microbial biomass carbon [MBC]; ratio MBC/C_{org}; ratio EC/MBC; potential respiration with glucose; potential ammonification with peptone; potential nitrification with ammonium sulphate and two model predicted values) for the study of the influence of grassing and harvest management. Zaman et al. (2002) used three parameters (MBC, microbial biomass N and extracellular enzyme activities) in different soil depths. Růžek et al. (2004) evaluated Cambisols (Inceptisols) and Luvisols (Alfisols) by six biological criteria (MBC and five ratios: MBC/C_{org}; EC/MBC; potential/basal respiration; potential/control ammonification; potential/control nitrification). Števlíková et al. (2003) evaluated two land managements on stagno-gleic Luvisol using MBC, MBC/C_{org},

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biologically releasable nitrogen [CA+CN] and intensity of nitrification [CN] after and before incubation. Wojewoda and Russel (2003) tested the impact of shelterbelts on soil properties and microbial activity through five criteria: MBC, BR, MBC/C_{org}, dehydrogenase activity, PA/CA.

The desiccation can play a very important role in changes of soil biological criteria. This treatment was studied for example by Nowak et al. (2000) in laboratory experiment where the influence of different dosages of herbicides on the activity of phosphatase and dehydrogenase in soil was determined. Reinecke et al. (2002) studied the impact of different herbicides on biological activity in several vineyard soils with and without cover crops in the Western Cape, South Africa. A comparison of microbial activity after the treatment with paraquat, simazine, glyphosate and glyphosate-terbutylazine mixture showed (with the exception of simazine) that the herbicide application had a positive influence.

The fallow is also a freqent subject of discussion; some authors treatise biological activities on such soils. The objective of the study of Okito et al. (2004) was to quantify the contribution of biological nitrogen fixation to groundnut (*Arachis hypogaea*) and velvet bean (*Mucuna pruriens*) crops using the N-15 natural abundance technique and to determine their residual effect and the effect of a natural fallow on the growth and N accumulation of two rustic maize varieties. Šantrůček et al. (2002) tested the composition of botanical species and agro-botanical groups (grasses, legumes, other dicotyledonous) from the third to the sixth year of vegetation on spontaneous fallow and manipulated fallows with 4 pure cultures of legumes, 4 pure cultures of grasses and their mixtures. The stands were cut one or three times per year or mulched ones or twice a year. The variants that were cut once a year had a significantly better plant cover.

The main aim of this study was to determine a variability of important microbiological criteria before and after desiccation by the glyphosate herbicide Roundup Bioactive.

MATERIAL AND METHODS

The experimental field (luvic chernozem, altitude 281 m, average precipitation 472 mm per year, average yearly temperature 9.3°C) is situated in Prague University campus area. Basic characteristics are presented in Table 1. Experimental field, formerly used in arable system with usual crop rotation (change in 1996), was divided into experimental plots (10 × 3 m) and was sown with 4 legumes (*Trifolium repens* L., *Medicago lupulina* L., *Lotus corniculatus* L., *Medicago media* Pers.), 4 grasses (*Bromus catharticus* Vahl, *Arrhenatherum elatius* (L.) Presl, *Festuca pratensis* Huds, *Dactylis aschersoniana* Graebn.) and with their mixtures. One part was cut three times a year with plant biomass removing and the second part was mulched one or two times a year (Table 5). Soil samples were collected during the years 2002–2004 at eleven sampling sites. In July 2003 and in June 2004 plots were desiccated by the glyphosate herbicide Roundup Biaktiv (5 and 4 l/hectare, respectively) and in August 2004 the plots were sown by ryegrass (*Lolium multiflorum* L.). Sampling was done before desiccation [BD] at November 2002, March 2003, April 2003, May 2003, June 2003 and July 2003 and after desiccation [D] at September 2003, October 2003, November 2003, April 2004 and August 2004.

Soil samples were taken from the profile (0 to 200 mm) using the sampler *Eijkelkamp*, they were transported in the cooling box (temperature 6 to 12°C), adjusted, sieved (mesh 2 mm) and stored in the refrigerator (4–6°C). 24 hours before analyses the samples were pre-incubated at the room temperature (22 ± 2°C).

The list of tests used for soil samples characterisation and microbial activity determination:

– texture: sand, silt, clay content (ISO 112 77) was determined by pipette method
– pH (H_2O), pH (0.2 mol/l KCl), 25 ml of reagent and 10 g of air-dried soil sample were shaken (15 minutes) and pH was determined after (over night) sedimentation
– total nitrogen (N_t) – Kjeldahl method
– organic carbon (C_{org}) – modified colorimetric determination in 600 nm (Sims and Haby 1971, Růžek et al. 2005)
– microbial biomass carbon (MBC) – re-hydration technique (RHD) with colorimetric determination in 590 nm (Blagodatskiy et al. 1987).
– K_2SO_4 extractable carbon (EC) – extraction with 0.5 mol/l K_2SO_4 (Vance et al. 1987, Růžek et al. 2005).
– respiration: basal (BR), potential with glucose (PR-G), potential with $(NH_4)_2SO_4$ (PR-N), potential with glucose and $(NH_4)_2SO_4$ (PR-NG) – interpherometric CO_2 detection (Novák and Apfelthaler 1964).
– actual content of $N\text{-}NH_4^+$ (ac $N\text{-}NH_4^+$)
– actual content of $N\text{-}NH_4^+$ with 8 days incubation (acI)

Table 1. Basic soil parameters (grassed luvic chernozem)

Textural and chemical parameters (*SD*)[25]		Soil microbial activities (*SD*)[25]		Other biological criteria (*SD*)[25]	
Sand[1] (0.063–2 mm)	17.93% (3.04)	BR[6, 7]	0.57 (0.18)	MBC[22]	621.38 (169.28)
Silt[1] (0.002–0.062 mm)	53.49% (3.62)	PR-N[6, 8]	1.06 (0.22)	EC[23]	44.52 (12.56)
Clay[1] (<0.002 mm)	28.58% (1.52)	PR-G[6, 9]	4.80 (1.75)	EC/MBC	7.55% (2.40)
Moisture[2]	14.73% (3.46)	PR-NG[6, 10]	18.72 (6.67)	MBC/C_{org}	3.14% (0.65)
pH (H_2O)	7.50 (0.19)	ac-N-NH_4^+[12]	16.76 (2.65)	Model MBC[24]	528.87 (46.52)
pH (KCl)	7.03 (0.15)	acI[11, 13]	16.80 (3.16)	MBC/Model MBC	117% (26.00)
E 400/E 600[3]	4.59 (0.18)	CA[11, 14]	14.94 (4.16)		
C_{org}[4]	1.97% (0.28)	PA-P[11, 15]	180.46 (51.70)		
N_t[5]	0.19% (0.02)	PA-NG[11, 16]	130.47 (26.90)		
C_{org}/N_t	10.49 (1.38)	ac-N-NO_3^-[17]	1.63 (1.32)		
		CN[18, 19]	2.41 (1.56)		
		PN[18, 20]	24.52 (13.27)		
		Arylsulphatase[21]	285.28 (127.16)		

[1]ISO 11277, [2]gravimetrically, [3]quality of humus substances, [4]colorimetrically, [5]Kjeldahl, [6]respiration, mg CO_2/h/100 g dry soil (Novák et Apfelthaler 1964), [7]basal, [8]potential with $(NH_4)_2SO_4$, [9]potential with glucose, [10]potential with $(NH_4)_2SO_4$ and glucose, [11]mg N-NH_4^+/24 h/100 g dry soil (Pokorná-Kozová et al. 1964), [12]actual content mg N-NH_4^+/100 g dry soil, [13]actual content with pre-incubation (8 days), [14]control ammonification, [15]potential ammonification with peptone, [16]potential ammonification with $(NH_4)_2SO_4$ and glucose, [17]actual content: mg N-NO_3^-/100 g dry soil, [18]nitrification, mg N-NO_3^-/8 days/100 g dry soil (Löbl et Novák, 1964), [19]control, [20]potential with $(NH_4)_2SO_4$, [21]mg *p*-nitrophenol/kg/h (Tabatabai et Bremner 1970), [22]microbial biomass carbon, mg/kg (Blagodatskiy et al. 1987), [23]0.5 mol/l K_2SO_4 extractable carbon (mg/kg), [24]Voříšek et al. 2002, [25]standard deviation

– control ammonification with 3 ml distilled water (CA)
– potential ammonification with 3 ml (33%) peptone (PA-P)
– potential ammonification with 3 ml 50% glucose solution) and 9.4% $(NH_4)_2SO_4$ solution) in ratio 1:1 (PA-NG) (Pokorná-Kozová et al. 1964)
– actual content of N-NO_3^- (ac-N-NO_3^-) (Löbl and Novák 1964)
– control nitrification with distilled water (CN), 8 days incubation
– potential nitrification (PN) with $(NH_4)_2SO_4$, 8 days incubation (Löbl and Novák 1964)
– arylsulphatase activity (three repetition and control): 1 g fresh soil was incubated with 4 ml acetate buffer and with 1 ml *p*-nitrophenylsulphate for 1 hour in 37°C (control without 1 ml *p*-nitrophenylsulphate); before filtration 1 ml *p*-nitrophenylsulphate was added to the control and 25 ml distilled water were added to both variants; 6 ml filtrate were taken and mixtured with 4 ml 0.5 mol/l NaOH; colorimetric determination in 420 nm (Tabatabai and Bremner 1970)

Following ratios were calculated:
– (MBC/C_{org}) × 100
– (EC/MBC) × 100

Results (110 soil samples before and 55 soil samples after desiccation) were statistically evaluated using analyses of variance (multiple range tests) including Fisher *LSD* method.

RESULTS AND DISCUSSION

Basic soil characteristics are presented in Table 1.

Effect of desiccation on soil organic carbon pool – C_{org}, MBC, EC (Tables 2 and 3)

Soil organic carbon (C_{org}): Chernozems are characterised by1.41% of C_{org}, in our experiment (Table 1) the average of all tested plots is higher (1.97%, *SD* 0.28). This result was influenced especially with high C_{org} level after desiccation (2.11%, *SD* 0.28) whereas before desiccation the average

Table 2. Biological criteria before and after desiccation

	BR[6, 7]	PR-N[6, 8]	PR-G[6, 9]	PR-NG[6, 10]	ac-N-NH_4^+[11, 12]	CA[11, 14]	acI[11, 13]
BD	0.63	1.10	4.76	19.77	17.29	15.56	18.09
D	0.48	0.97	4.88	16.61	15.70	13.69	14.23
LSD_{95}[a] $d_{\alpha\ min}0.05$	0.06*	0.08*	0.66	2.45*	0.96*	1.54*	0.98*
LSD_{99}[a] $d_{\alpha\ min}0.01$	0.08**	0.11**	0.87	3.24**	1.27**	2.03**	1.28**
	PA-P[11, 15]	PA-NG[11, 16]	ac-N-NO_3^-[17]	CN[18, 19]	PN[18, 20]	arylsulphatase	
BD	180.18	141.08	1.35	2.19	25.09	312.64	
D	181.01	109.23	2.18	2.86	23.36	257.92	
LSD_{95}[a] $d_{\alpha\ min}0.05$	19.53	8.42*	0.48*	0.58*	5.00	47.16*	
LSD_{99}[a] $d_{\alpha\ min}0.01$	25.78	11.11**	0.63**	0.76**	6.61	62.38	

[6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20] see Table 1; [a]Fisher's Least Significant Difference; $^{**}P < 0.01$, $^{*}P < 0.05$; BD before desiccation by Roundup Biaktiv (averages), D after desiccation by Roundup Biaktiv (averages)

from all plots was 1.83% (*SD* 0.28); the difference (Table 3) was significant ($P < 0.01$).

Soil microbial biomass carbon (MBC): Microbial biomass is the most active part of soil organic matter and ratio MBC/C_{org} was stressed (Insam and Domsch 1988) as a very important indicator of soil microbial status. According to our former experience the range of this ratio is 2.5–4.5% at arable and grassed soils, for chernozems (mollisols) the average is 2.97%. Determination of MBC is a widely used test for evaluation of environmental and antropogenic influences on living part of soils (Filip 2001, etc.). Expected level of MBC in luvic chernozem is 473.7 mg/kg dry soil (*SD* 44.0). In our experiment the average was 621.38 mg/kg dry soil (*SD* 169), this level was composed from 510.64 mg/kg dry soil before and 732.13 mg/kg dry soil after desiccation; the difference was significant ($P < 0.01$).

K_2SO_4 extractable carbon (EC): EC is a trophically easily usable organic carbon of microbial origin and so its low content is a sign of active microbial metabolism (Škoda et al. 1997). Chernozems (Voříšek et al. 2002) were slightly more active in the use of EC (45 mg/kg dry soil) than for example cambisols (48 mg/kg dry soil), in contrast luvisols (Růžek et al. 2004) were significantly more active (40 mg/kg dry soil). There was no significant difference before and after desiccation in EC.

Ratio MBC/C_{org}: The ratio gives the information about the level of metabolic active carbon in the total soil organic matter; usual level of luvic chernozem is 3.47% (*SD* 0.27). The ratio of our tested plots (Table 3) was significantly different ($P < 0.01$) before (2.79%) and after (3.48%) desiccation.

Effect of desiccation on C_{org} mineralization (CO_2 respiration)

The average level of basal (control) respiration (Novák et Apfeltaler 1964) of top-soil (0–200 mm) was 0.50 ± 0.26 mg CO_2/h/100 g dry soil, potential respiration with ammonium sulphate was slightly higher (110% of basal respiration) in the soils where nitrogen was not a limiting factor. According to our experience potential respiration with glucose in the microbial active soils reaches approximately 700% of basal respiration.

There was a statistically significant difference ($P < 0.01$) in control respiration before (0.63 mg

Table 3. Other criteria before and after desiccation

	Moisture[2] (%)	E 400/E 600[3]	C_{org}[4]	N_t[5]	C_{org}/N_t	MBC[22]	EC/MBC (%)	Model MBC[24]	MBC/Model MBC (%)	EC[23]	MBC/C_{org} (%)
BD	15.29	4.51	1.83	0.13	10.14	510.64	8.53	509.70	99.88	42.50	2.79
D	13.61	4.67	2.11	0.20	10.85	732.13	6.57	548.04	133.26	46.54	3.48
LSD_{95}[a] $d_{\alpha\ min}0.05$	1.27*	0.07*	0.11*	0.01*	0.57*	54.35*	0.94*	18.06*	8.65*	5.28	0.24*
LSD_{99}[a] $d_{\alpha\ min}0.01$	1.68**	0.09**	0.14**	0.01	0.75	72.02**	1.24**	23.92**	11.46**	7.00	0.31**

[2, 3, 4, 5, 22, 23, 24] see Table 1; [a]Fisher's Least Significant Difference; $^{**}P < 0.01$, $^{*}P < 0.05$; BD before desiccation by Roundup Biaktiv (tests averages), D after desiccation by Roundup Biaktiv (tests averages)

CO_2/h/100 g dry soil) and after desiccation (0.48 mg CO_2/h/100 g dry soil), as shown in Table 2. There was a significant difference ($P < 0.01$) between soils before (1.10 mg CO_2/h/100 g dry soil) and after (0.97 mg CO_2/h/100 g dry soil) desiccation in potential respiration with $(NH_4)_2SO_4$ (Table 2). Potential respiration (Table 2) with glucose was determined at the level 4.80 mg CO_2/h/100 g dry soil (*SD* 1.75); it was higher than the usual level of 3.79 mg CO_2/h/100 g dry soil (*SD* 0.25). As for this criterion there was not a significant difference before (4.76 mg CO_2/h/100 g dry soil) and after (4.88 mg CO_2/h/100 g dry soil) desiccation (Table 2). It could be expected because of the increasing available C content in the soil from the plants decay.

In the potential respiration with $(NH_4)_2SO_4$ and glucose there was a significant difference ($P < 0.01$) before and after desiccation (19.77 and 16.61 mg CO_2/h/100 g dry soil, respectively).

Effect of desiccation on N_{org} mineralization (ammonification) and nitrification

Actual content of N-NH_4^+ without (ac-N-NH_4^+) and with pre-incubation (acI): The usual level 19.19 mg N-NH_4^+/100 g dry soil (*SD* 1.30) was not assessed in our experiment, it was lover than normal (16.76 mg N-NH_4^+/100 g dry soil, *SD* 2.65) (Table 2). There was a significant decrease ($P < 0.01$) of ac-N-NH_4^+ before (17.29 mg N-NH_4^+/100 g dry soil) and after (15.70 mg N-NH_4^+/100 g dry soil) desiccation. In acI there was a significant difference in soils before and after desiccation ($P < 0.01$).

Control ammonification: There was a significant difference ($P < 0.01$) before and after desiccation (Table 2).

Potential ammonification with peptone (PA-P), with $(NH_4)_2SO_4$ and glucose (PA-NG): There were no significant differences in PA-P between soils

Table 4. Correlation coefficients between arylsulphatase activity and other tests

BR	PR-N[6, 8]	PR-G[6, 9]	PR-NG[6, 10]	ac-N-NH_4^+[11, 12]	CA[11, 14]	PA-P[11, 15]	PA-NG[11, 16]	N-NO_3^- (ac)[17]	CN[18, 19]
0.6313***	0.4824***	0.3071**	0.2882**	0.4319***	0.2444*	0.1084	0.2178*	0.0380	–0.0152
PN[18, 20]	E 400/ E 600[3]	C_{org}[4]	N_t[5]	C_{org}/N_t	MBC[22]	EC/MBC	Model MBC[24]	MBC/Model MBC	EC[23]
0.4514***	0.0960	–0.0157	0.0896	0.0698	–0.0505	0.2579*	0.0565	–0.0724	0.1729

[3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 14, 15, 16, 17, 18, 19, 20, 22, 23, 24] see Table 1; $^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$

Table 5. Ranking based on sixteen biological criteria[1] before and after desiccation

Management	Plants	Plot number	BD	Management	Plants	Plot number	D
Two mulching per year	legumes	9	1	One mulching per year	legumes	7	1
One mulching per year	legumes	7	2	Two mulching per year	legumes	9	2
Two mulching per year	mixtures	12	3	One mulching per year	grasses	6	3
One mulching per year	mixtures	11	4	One mulching per year	mixtures	11	4
One mulching per year	grasses	6	5	Two mulching per year	mixtures	12	5
Two mulching per year	grasses	8	6	Spontaneous fallow		5	6
Spontaneous fallow		5	7	Two mulching per year	grasses	8	7
Three cutting per year	mixtures	10	8	Three cutting per year	legumes	3	8
Three cutting per year	legumes	3	9	Three cutting per year	mixtures	10	9
Three cutting per year	grasses	2	10	Three cutting per year	grasses	2	10
Black fallow		1	11	Black fallow		1	11

[1]sixteen biological criteria:

1 BR	5 ac-N-NH_4^+	9 PA-NG	13 arylsulphatase
2 PR-N	6 acI	10 ac- N-NO_3^-	14 MBC
3 PR-G	7 CA	11 CN	15 EC/MBC
4 PR-NG	8 PA-P	12 PN	16 MBC/C_{org}

BD ranking before desiccation by Roundup Biaktiv, D ranking after desiccation by Roundup Biaktiv

before and after desiccation (180.18 and 181.1 mg N-NH_4^+/24 h/100 g dry soil, respectively). Our data also confirmed that in soils with neutral pH there is a close relation between high microbial biomass C content and middle potential ammonification. There was a significant difference in PA-NG before and after desiccation ($P < 0.01$) (Table 2).

Actual content of NO_3^- (ac-N-NO_3^-): According to our experience the usual level in chernozem is 2.63 mg N-NO_3^-/100 g dry soil (*SD* 4.07). In our experiment we found a lover content than normal (1.63 mg N-NO_3^-/100 g dry soil, *SD* 1.32); ac-N-NO_3^- significantly increased in the soil after desiccation because of decreased immobilization by the plants (Table 2).

Control nitrification: In this parameter we assessed a significant difference ($P < 0.01$) before and after desiccation.

Potential nitrification with $(NH_4)_2SO_4$ (PN): This test is very often used as an indicator of soil microbial activity. There was no difference in PN before and after desiccation because available N was not immobilized by plants (Table 2). The usual level in chernozem is 12.11 mg N-NO_3^-/8 days/100 g dry soil (*SD* 2.93) but in our tests we measured 24.52 mg N-NO_3^-/8 days/100 g dry soil (*SD* 13.27) (Table 2). A similar tendency (high activity) was in glucose mineralization tested by PR-G. These positive changes in microbial metabolism are surely connected with neutral pH KCl (7.3).

Desiccation and arylsulphatase activity

Before desiccation we determined higher ($P < 0.05$) soil arylsulphatase activity than after it (312.64 and 257.92 mg *p*-nitrophenol/kg/h, respectively). The correlation coefficients between arylsulfatase activity and other tests are given in Table 4. Lu-Qin et al. (2003) presented a significant correlation between arylsulphatase activity and C_{org}; in our results this was not confirmed ($r = -0.0157$). Dedourge et al. (2004) also informed about a significant correlation between arylsulphatase activity and MBC. Neither this correlation was confirmed in our tests ($r = -0.0505$).

Biological status before and after desiccation; ranking based on 16 biological criteria

Sixteen biological criteria (MBC, BR, PR-N, PR-G, PR-NG, ac-N-NH_4^+, acI, CA, PA-P, PA-NG, ac-N-NO_3^-, CN, PN, arylsuphatase activity and

two ratios: MBC/C_{org}; EC/MBC) were used for soil biological status evaluation before and after desiccation (Table 5). 11 plots with different management and plant cover were evaluated and their ranking based on the above-mentioned criteria was determined both before and after desiccation. The black fallow ranked the worst both before and after desiccation. One or two mulching variants were the best also before and after desiccation. The cut plots ranked on the 8–10 positions. The spontaneous fallow reached the middle position. Similar results were published by Voříšek et al. (2002).

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