Soil biological activity of mulching and cut/harvested land set aside

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ABSTRACT

Formerly arable luvic chernozem set aside for ten years (1997–2006) with different herbaceous cover (grass, legumes and their mixtures) and agronomical practices (mulching and cut/harvesting) was studied. The experimental plot was maintained as black, spontaneous and controlled fallows from 1997 until July 2003 (BD period). In July 2003 the plots were desiccated by glyphosate herbicide and were run as a black fallow until August 2004 (AD). The last period (AG) was characterized by monoculture of Italian ryegrass cut/harvested twice a year until October 2006. The experimental soils were characterized with 18 parameters. Mulched plots in all periods (BD, AD and AG) were evaluated as highly microbial active plots. The black fallow (BD) permanently maintained by glyphosate herbicide was the lowest in biological parameters. The desiccation caused a highly significant increase ($P < 0.01$) of nitrates in topsoil, but in following period (AG) their significant decrease was detected. Desiccation enhanced carbon immobilization into microbial cells especially on mulched and cut/harvested sites (BD, AD). Due to mulching accumulation of soil organic matter highly significantly increased ($P < 0.01$). This induced a highly significant ($P < 0.01$) increase in the basal respiration (AD, AG) as the soil organic matter accumulated in the period BD was intensively mineralized.

Keywords: fallow; desiccation; Roundup; microbial biomass; K_2SO_4 extractable carbon; respiration; nitrification; ammonification; metabolic quotient

Microbial biomass, composition of microflora, mineralization and synthesising processes are ecologically important soil characteristics (Filip 2001) that play a key role in different ecologically important soil functions. The fallow is also very often the subject of discussion; some authors treatise biological activities of such soils.

Many methods determining both quantity of microorganisms and their activities can be used for the characterization of soil biological quality. Voříšek et al. (2002) used three or eight parameters [microbial biomass carbon (MBC); ratio MBC/C_{org}; ratio E_C/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.

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 four pure cultures of legumes, four 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 which were cut once a year had significantly better plant cover.

The main aim of this study was to determine variability of important microbial criteria of mulched and cut/harvested land set aside before and after desiccation by glyphosate herbicide Roundup Biaktiv and after grassing by Italian ryegrass.

MATERIAL AND METHODS

The experimental field (luvic chernozem, altitude 281 m, average precipitation 472 mm per

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year, average year temperature 9.3°C) is situated at Czech University of Life Sciences campus area. Basic characteristics are presented in the Table 1. Experimental field (1800 m^2), formerly (change in 1996) used in arable system with usual crop rotation, was divided into experimental plots (10 × 3 m) and was sown with four pure culture legumes (*Trifolium repens* L., *Medicago lupulina* L., *Lotus corniculatus* L., *Medicago media* Pers.), four pure culture grasses (*Bromus catharticus* Vahl, *Arrhenatherum elatius* L. Presl, *Festuca pratensis* Huds., *Dactylis aschersoniana* Graebn.) and their respective mixtures. One part of experimental plot was cut three times a year with plant biomass removing and the second part was mulched by AS 27/2 Enduro machine one or two times a year (Table 2). Soil samples were collected during the years 2002–2006 at eleven sampling sites. In July 2003 and in June 2004 all experimental plots were desiccated by glyphosate herbicide Roundup Biaktiv (5 and 4 l/ha) and in September 2004 all experimental plots were grassed with ryegrass (*Lolium multiflorum* L.). Sampling was done before desiccation (BD; 110 soil samples) from November 2002 until July 2003, after desiccation (AD; 55 soil samples) from September 2003 until August 2004 and after grassing with Italian ryegrass (AG; 77 soil samples) from October 2004 until October 2006. Soil samples were taken from the profile (0–200 mm) using Eijkelkamp sampler. The soil samples were transported in the cooling box (temperature 6–12°C), adjusted, sieved (mesh 2 mm) and stored in refrigerator (4–6°C). 24 h before analysis the samples were pre-incubated at the room temperature (22 ± 2°C).

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

(a) texture: sand, silt, clay content (ISO 112 77) was determined by pipette method;

(b) pH (H_2O), pH (0.2 mol/l KCl); 25 ml of reagent and 10 g of air-dried soil sample were shaken (15 min) and pH was determined after overnight sedimentation with amplified electrode with Hanna instruments;

(c) total nitrogen (N_t) – Kjeldahl method;

(d) soil organic carbon (C_{org}) – colorimetric determination in 600 nm (Šims and Haby 1971, modified by Růžek et al. 2005);

(e) microbial biomass carbon (MBC) – rehydration technique (RHD) with colorimetric determination at 590 nm (Blagodatskiy et al. 1987, modified by Růžek et al. 2005);

(f) K_2SO_4 extractable carbon (E_C) – extraction with 0.5 mol/l K_2SO_4 (Vance et al. 1987, modified by Růžek et al. 2005);

(g) respiration: basal (BR), potential with glucose (PR-G) and potential with $(NH_4)_2SO_4$ (PR-N) – interpherometric CO_2 detection (Novák and Apfelthaler 1964);

(h) control ammonification (CA), 24 h incubation;

(i) potential ammonification with 33% peptone (PA-P) (Pokorná-Kozová et al. 1964);

Table 1. Basic soil characteristics

Textural and chemical parameters (SD)[18]		Soil microbial activities (SD)[18]		Other biological criteria (SD)[18]	
Sand[1] (0.063–2 mm)	20.47% (2.76)	BR[6, 7]	0.48 (0.15)	MBC[16]	579.85 (114.9)
Silt[1] (0.002–0.062 mm)	52.69% (4.17)	PR-N[6, 8]	1.04 (0.20)	E_C[17]	40.11 (13.41)
Clay[1] (< 0.002 mm)	26.84% (2.19)	PR-G[6, 9]	4.25 (1.37)	E_C/MBC	7.33% (2.54)
Moisture[2]	15.95% (3.39)	CA[10, 11]	16.96 (3.39)	MBC/C_{org}	3.27% (0.45)
pH (H_2O)	7.6 (0.3)	PA-P[10, 12]	220.4 (50.0)		
pH (KCl)	6.7 (0.2)	CN[13, 14]	1.82 (1.41)		
E 400/E 600[3]	4.82 (0.40)	PN[13, 15]	24.37 (12.76)		
C_{org}[4]	1.54% (0.27)				
N_t[5]	0.19% (0.02)				
C_{org}/N_t	9.7 (1.4)				

[1]ISO 11277 (1998); [2]gravimetrically; [3]quality of humus substances; [4]colorimetrically; [5]Kjeldahl method; [6]respiration, mg CO_2/h/100 g dry soil; [7]basal; [8]potential with $(NH_4)_2SO_4$; [9]potential with glucose; [10]mg N-NH_4^+/24 h/100 g dry soil; [11]control ammonification; [12]potential ammonification with peptone; [13]nitrification, mg N-NO_3^-/8 days/100 g dry soil; [14]control; [15]potential with $(NH_4)_2SO_4$; [16]microbial biomass carbon (mg/kg); [17]0.5 mol/l K_2SO_4 extractable carbon (mg/kg); [18]standard deviation

Table 2. List of plots

BF	black fallow
3CG	grasses, cutting three times per year with plant biomass removing
3CL	legumes, cutting three times per year with plant biomass removing
SF	mulching, spontaneous fallow
1MG	grasses, mulching one time per year
1ML	legumes, mulching one time per year
2MG	grasses, mulching two times per year
2ML	legumes, mulching two times per year
3CM	mixtures, cutting three times per year with plant biomass removing
1MM	mixtures, mulching one time per year
2MM	mixtures, mulching two times per year

(j) control nitrification (CN), 8 days incubation;
(k) potential nitrification (PN) with $(NH_4)_2SO_4$, 8 days incubation (Löbl and Novák 1964).

All microbial activity tests were run at 28°C.

Following six ratios were calculated:
(1) $(MBC/C_{org}) \times 100$;
(2) $(E_C/MBC) \times 100$;
(3) PR-G/BR;
(4) PR-N/BR;
(5) PA-P/CA;
(6) PN/CN.

Statistical evaluation was attained with analysis of variance (Multiple range test) including Fisher's Least Significant Difference (LSD) determination.

RESULTS AND DISCUSSION

This work includes the results of a five-year (2002–2006) observation of eleven plots (Tables 1 and 2) on luvic chernozem that was set aside in 1997 during three periods: before desiccation (BD), after desiccation (AD) and after grassing with Italian ryegrass (AG).

Set aside variants and their soil biological activity. Evaluation according to eleven criteria (Table 3) describes mulched plots in all periods (BD, AD and AG) as the plots with high biological activities. From a microbiological point of view a black fallow maintained in 1997–2004 with glyphosate herbicide Roundup Biaktiv (4 l per hectare three times a year) was the worst variant in all three periods. The changes in botanical composition in each variant evaluated by the mass method were published by Šantrůček et al. (2002), Svobodová et al. (2004) and Brant et al. (2006). According to these authors, the plots, which were cut/harvested three times a year, were the best variant regarding their botanical composition.

The influence of the desiccation by Roundup Biaktiv herbicide and grassing with Italian ryegrass on the soil biological activity. The desiccation dramatically affected increase of nitrates in topsoil with a statistical significance ($P < 0.01$). Plant cover returning to the site (grassing with Italian ryegrass) on the other hand evoked a decrease of nitrates in soil. A cumulative overall yield in 1998–2000 (before desiccation) was the largest one on the following plots: 2MM (twice a year mulched mixtures), 3CM (three times a year cut mixtures) and 2ML (twice a year mulched legumes). After grassing with Italian ryegrass (twice a year cut/harvested in 2004–2006), the highest cumulative overall yield (larger than 18 t/ha) was observed on plots 1ML and 2ML. As mentioned above, 1ML and 2ML plots were according eleven

Table 3. Eleven criteria used for evaluation

1. Soil moisture – gravimetrically (%)
2. Carbon of soil organic matter C_{org} (%)
3. Total nitrogen N_t (Kjeldahl method; %)
4. Microbial biomass carbon (MBC; mg/kg dry soil)
5. Ratio E_C[1]/MBC
6. Ratio MBC/C_{org}
7. Metabolic quotient (qCO_2) = (BR[2] × $k_{C\text{-}CO_2}$[3]/MBC) × 100
8. Ratio PR-N[4]/BR[2]
9. Ratio PR-G[5]/BR[2]
10. Ratio PA-P[6]/CA[7]
11. Ratio PN[8]/CN[9]

[1]0.5 mol/l K_2SO_4 extractable carbon; [2]basal respiration (mg CO_2/h/kg dry soil); [3]0.2727; [4]potential respiration with $(NH_4)_2SO_4$; [5]potential respiration with glucose; [6]potential ammonification with peptone; [7]control ammonification; [8]potential nitrification; [9]control nitrification

criteria the plots with the highest biological activity. It is a question whether the highest production of above ground biomass is a suitable criterion for set aside sites. However, the overall success of 1ML and 2ML variants is not to be diminished by this doubtful criterion.

The desiccation of the plant cover by Roundup Biaktiv herbicide significantly affected biological properties of the soil. High significant increase was in potential respiration with glucose (PR-G) and control nitrification (CN). The increase of nitrates after the desiccation can be explained by the decrease in their intake that is evoked by the absence of photosynthetic active plants. A significant increase was also noted in basal respiration (BR). A significant decrease was determined in control ammonification (CA). The plant cover desiccation significantly supported the accessible carbon immobilization into microbial cells, which was mirrored by two outputs: the above-mentioned potential respiration with glucose and the MBC/C_{org} ratio. These two outputs documented a pronounced immobilization on mulched as well as on cut/harvested plots.

The grassing with Italian ryegrass resulted in a high significant decrease in control nitrification (CN), potential nitrification (PN) and potential respiration with glucose addition (PR-G). A high significant increase was observed in potential ammonification (PA-P) and basal respiration (BR). Due to the agronomic practices before desiccation (1997–2003), when mulching was predominant (once or twice a year), the accumulation of soil organic matter was supported. This caused also a significant increase of basal respiration (Table 4) at AD period as well as at AG period when the accumulated soil organic matter was intensively mineralized due to the Italian ryegrass cut/harvest twice a year. The situation at AG period would resemble the BD period if the Italian ryegrass were mulched. Cumulative overall yield at AG period on all plots ranged from 8 to 18 tons per hectare.

Basal respiration (BR). A high significant increase in CO_2 production at the last period (AG) is unfavourable from an ecological point of view. Italian ryegrass cut/harvested twice per year in 2005–2006 encouraged the mineralization of soil organic matter (SOM) more than the plant biomass mulching. Mixture of Italian ryegrass with legumes mulched once a year would be a better option, also in regard to contamination with weeds. The measured values of basal respiration (Table 4) were in the range typical (Růžek et al. 2006) for grass-covered luvic chernozem: 0.38–0.62 mg CO_2/h/100 g of dry soil. However, 55% of the experimental sites showed respiration values in the upper margin of this interval. In general, the average was lowered at some test plots: black fallow, mulched mixtures, or cut/harvested grass. On the other hand, cut legumes, a spontaneous fallow and mulched grass as well as legumes exceeded this average (Nováková and Voříšek 2006).

Potential respiration with ammonia-N addition (PR-N). A usual high respiratory response to the $(NH_4)_2SO_4$ addition (at the level of 137 to 239% of the basal respiration) proved that the

Table 4. Biological criteria before desiccation (BD), after desiccation (AD) and after grass by Italian ryegrass (AG)

	Mineralization of C_{org} (respiration)			Mineralization of N_{org} (ammonification)		Oxidation of NH_4^+ (nitrification)		MBC[8]
	BR[1]	PR-N[2]	PR-G[3]	CA[4]	PA-P[5]	CN[6]	PN[7]	
BD (n = 110)	0.38	1.05	3.03	18	190.33	1.63	24.84	435.22
AD (n = 55)	0.45	0.95	5.72	16.21	191.94	2.69	27.76	732.13
AG (n = 77)	0.62	1.11	4.00	16.66	278.99	1.13	20.51	572.21
[9]LSD_{95} d_{min}0.05*	0.07	0.13	0.54	1.69	20.17	0.60	5.42	45.93
[9]LSD_{99} d_{min}0.01**	0.09	0.17	0.72	2.22	26.59	0.79	7.16	60.50
Statistically significant differences of parameters	BD/AD* AD/AG** BD/AG**	AD/AG*	BD/AD* AD/AG** BD/AG**	BD/AD*	AD/AG** BD/AG**	BD/AD** AD/AG**	AD/AG**	BD/AD** AD/AG** BD/AG**

[1]basal respiration; [2]potential respiration with $(NH_4)_2SO_4$; [3]potential respiration with glucose; [4]control ammonification; [5]potential ammonification; [6]control nitrification; [7]potential nitrification; [8]microbial biomass carbon; [9]Fisher's Least Significant Difference (*P < 0.05; **P < 0.01)

entire experimental site (Table 4) was lacking physiologically available nitrogen (PAN) for SOM mineralization throughout the whole observation period (BD, AD and AG). PAN shortage was even registered on plots where legumes were grown before the desiccation. Twice a year mulched pure grass culture was relatively the most favourable variant. The highest values of PAN were recorded on plots with the lowest amount of nitrates. On the other hand the lowest values of PAN were found on the plots with the highest amounts of nitrates (former black fallow and mulched legumes and mixtures). According to Růžek et al. (2003) standard respiratory response is less than or equal to 110% of the basal respiration and it is a sign of PAN sufficiency. However, such values were not recorded on the experimental sites throughout the entire observation period (Table 6). A highly statistically significant correlation ($P < 0.001$) was observed between the control nitrification and ratio PR-N/BR in the BD period ($r = 0.8054$) and AG period ($r = 0.5843$), respectively. A negative relationship was observed between PAN and nitrates. However, this relationship was not confirmed during the AD period.

Potential respiration with glucose addition (PR-G). The high level of PR-G during the AD period (Table 4) is probably connected to physiologically available carbon (PAC) missing in topsoil at AD period. However, in the following AG period this unfavourable situation was improved with a high significance due to an input of PAC in the form of root exudates. From the evaluation of the BD period it is possible to claim that if a mixture of grasses and legumes cut or mulched twice a year were grown instead of twice a year cut Italian ryegrass, the amount of the PAC would be even greater. The usual value of the PR-G/BR ratio for chernozems (Růžek et al. 2006) is in the range of 8.82–9.28. Favourable values of this ratio were detected in the last AG period throughout the entire observation site (Table 6). On the other hand very unfavourable values were observed in the AD period also throughout the entire site. The highest value of the ratio 15.57 (Table 6), which was observed in the once a year mulched legumes, was rather extreme. The desiccation supported the immobilization of carbon into microbiological cells. This was most strongly expressed in legumes mulched once a year.

Control ammonification (CA). The results showed (Table 6) that the plots with plant cover mulched twice a year contain more ammonia-N than the plots mulched once. The least content of ammonia-N was detected on the black fallow throughout the entire observation periods (BD, AD and AG).

Potential ammonification with peptone (PA-P). The presence of PAN in soil very likely leads to lower than average values of this parameter. According to Růžek et al. (2006) the average values in chernozem are 184.01 ± 47.20 mg $N\text{-}NH_4^+$/24 h/100 g dry matter. Legumes that were on the observation site at BD period maintained this parameter in the above-mentioned range. However, this was changed at AG period, as legumes were missing in the pure culture of Italian ryegrass. The reaction of soil microflora to the addition of peptone significantly increased. Elliott et al. (1996) connected the values of the potential ammonification with soil quality. The results of our study confirm their opinion.

The increasing values of the ratio of the potential ammonification with peptone and the control ammonification (PA-P/CA) also refer to the absence of legumes. The highest value (23.13) was found at AG period on the plots previously maintained as a black fallow (Table 6). On the other hand, it is possible to state that even twenty applications of systematic glyphosate herbicide Roundup Biaktiv on a black fallow at periods BD and AD in the recommended dose of 4–5 l/ha did not negatively

Table 5. Metabolic quotient before desiccation (BD), after desiccation (AD) and after grass with Italian ryegrass (AG)

		BD (n = 110)	AD (n = 55)	AG (n = 77)
qCO_2[1]	average (SD)[2]	0.24 (0.08)[1]	0.17 (0.04)[1]	0.30 (0.09)[1]
	the highest plot	0.27 (SF)	0.21 (SF)	0.36 (BF)
	the lowest plot	0.20 (1ML)	0.15 (1ML)	0.26 (1MM)
	[3]LSD_{95} d_{min}0.05 (LSD_{99} d_{min}0.01)	0.06 (0.08)	0.06 (0.08)	0.10 (0.13)
	difference between plots	0.07*	0.06	0.10*

[1]mg $C\text{-}CO_2$/h/100 mg MBC; [2]standard deviation; [3]Fisher's Least Significant Difference ($^*P < 0.05$; $^{**}P < 0.01$)

Table 6. Other criteria (ratios) before desiccation (BD), after desiccation (AD) and after grass with Italian ryegrass (AG)

		BD (n = 110)	AD (n = 55)	AG (n = 77)
	average (SD)[1]	2.84 (0.57)[1]	3.86 (0.60)[1]	3.10 (0.45)[1]
	the highest plot (+)	3.30 (3CL)[3]	4.49 (1ML)[4]	3.52 (3CL)[3]
(MBC/C_{org}) × 100	the lowest plot	2.34 (SF)[5]	3.21 (BF)[6]	2.61 (BF)[6]
	[2]LSD_{95} d_{min}0.05 (LSD_{99} d_{min}0.01)	0.41 (0.54)	0.67 (0.90)	0.34 (0.46)
	difference between plots	0.96**	1.28**	0.91**
	average (SD)[1]	9.44 (2.94)[1]	6.57 (2.31)[1]	5.97 (2.38)[1]
	the highest plot	11.01 (2MM)[7]	8.78 (3CL)[3]	7.01 (2MM)[7]
(E_C/MBC) × 100	the lowest plot (+)	7.71 (2MG)[8]	5.16 (BF)[6]	4.54 (2MG)[8]
	[2]LSD_{95} d_{min}0.05 (LSD_{99} d_{min}0.01)	2.33 (3.08)	3.45 (4.63)	2.37 (3.15)
	difference between plots	3.30**	3.62*	2.48*
	average (SD)[1]	1.74 (0.36)[1]	2.06 (0.31)[1]	1.89 (0.35)[1]
	the highest plot	2.39 (BF)[6]	2.25 (2MM)[7]	2.08 (2ML)[9]
PR-N/BR	the lowest plot (+)	1.37 (3CG)[10]	1.85 (2MG)[8]	1.64 (2MG)[8]
	[2]LSD_{95} d_{min}0.05 (LSD_{99} d_{min}0.01)	0.69 (0.98)	0.59 (0.80)	0.35 (0.47)
	difference between plots	1.02**	0.40	0.44*
	average (SD)[1]	8.62 (3.92)[1]	12.93 (4.30)[1]	7.05 (2.50)[1]
	the highest plot	10.67 (BF)[6]	15.57 (1ML)[4]	8.61 (BF)[6]
PR-G/BR	the lowest plot (+)	6.81 (2MG)[8]	10.60 (3CM)[11]	6.10 (3CM)[11]
	[2]LSD_{95} d_{min}0.05 (LSD_{99} d_{min}0.01)	3.35 (4.43)	6.52 (8.76)	2.72 (3.62)
	difference between plots	3.86*	4.97	2.51
	average (SD)[1]	11.13 (4.46)[1]	11.73 (4.15)[1]	17.31 (3.65)[1]
	the highest plot (+)	12.74 (BF)[6]	12.92 (2MM)[7]	23.13 (BF)[6]
PA-P/CA	the lowest plot	10.40 (2MM)[7]	10.36 (1MG)[12]	12.76 (2MG)[8]
	[2]LSD_{95} d_{min}0.05 (LSD_{99} d_{min}0.01)	3.90 (5.16)	6.63 (8.91)	2.70 (3.60)
	difference between plots	2.34	2.56	10.37**
	average (SD)[1]	24.36 (18.99)[1]	13.68 (9.66)[1]	28.32 (22.03)[1]
	the highest plot (+)	38.56 (2MG)[8]	22.70 (2ML)[9]	43.11 (1ML)[4]
PN/CN	the lowest plot	13.80 (BF)[6]	8.11 (SF)[5]	9.10 (BF)[6]
	[2]LSD_{95} d_{min}0.05 (LSD_{99} d_{min}0.01)	18.84 (24.98)	14.26 (19.15)	27.39 (36.59)
	difference between plots	24.76*	14.59*	34.01*

[1]standard deviation; [2]Fisher's Least Significant Difference (*P < 0.05; **P < 0.01); [3, 4, 5, 6, 7, 8, 9, 10, 11, 12] see Table 1

affect the ammonification of soil microflora. On the contrary, it seems that the application of the systematic glyphosate herbicide Roundup Biaktiv enhanced the ammonification of soil microflora. Kara et al. (2004) achieved the same conclusion on sandy alkaline soil with the herbicide Topogard 50 WP (3 kg/ha).

Control nitrification (CN). Regarding a real possibility of leaching of nitrates into underground waters as well as into surface waters, especially during torrential rains, the situation at AD period was ecologically dangerous because of the high content of nitrates in topsoil. The AD period thus should be considerably shorter than during this experiment, where the ryegrass sowing was delayed due to extreme draught from August 2003 to November 2003 as well as in April 2004. On the other hand, it was the draught that decreased the danger of leaching of nitrates. The risk month during the AD period was definitely June 2004 with above-average rainfall, where the considerable weed contamination of the entire observation site was solved by the second desiccation (Roundup Biaktiv; 4 l/ha). Topsoil under three-times-a-year-cut mixtures had the lowest content of nitrates of all periods (BD, AD and AG). The second lowest content was found in topsoil under cut and mulched grasses. The combination of pure cultures of grasses and mulching occurs favourable regarding nitrates content in set-aside soil (Voříšek et al. 2002). The highest contents of nitrates were determined by the same authors on a black fallow, under cut and mulched legumes and under mulched mixtures.

Potential nitrification (PN). The highest values of PN were observed on plots mulched twice a year. The lowest values were found on plots that were cut/harvested three times a year and on the plots with a black fallow. The ability of oxidation of ammonia-N at AG period decreased with high significance. This is very likely due to the agronomic practices (Italian ryegrass cut/harvested twice a year). Values of PN at BD period were relatively low on the majority of plots. In contrast, the highest values were under twice-a-year-mulched pure cultures of grasses. Fiala and Gaisler (2000) also found a connection between nitrification and agronomic practices in their similarly designed experiment.

Microbial biomass carbon (MBC). MBC measured by rehydration (RHD) technique according to Blagodatskiy et al. (1987) (k = 4.00) in a set-aside luvic chernozem (0–200 mm) reached the values from 343.3 to 911.7 mg/kg. Kiem and Kandeler (1997) studied the MBC using chloroform fumigation extraction method (CFE) in a set of 35 locations where chernozems predominated (12) and they reached 128–515 mg/kg. MBC and qCO_2 (calculated as the BR/MBC ratio describing the production of carbon dioxide per 100 mg MBC) are important criteria of soil quality. Other ratios (MBC/C_{org} and E_C/MBC) describe carbon immobilization into microbial cells and a metabolic status of soil microbial associations, respectively. A significant difference (Tables 5 and 6) among the variants was observed in all mentioned criteria in all three periods (BD, AD and AG).

Metabolic quotient (qCO_2). Metabolic quotient (qCO_2) is defined as carbon dioxide production per a unit of microbial biomass per a time unit. According to our experience, very low values (under 0.15 mg $C\text{-}CO_2$/h/100 mg MBC) are sign of a very low mineralization activity. Values between 0.15 and 0.33 (Růžek et al. 2006) are characteristic as standard for main soil taxonomical units. High values indicate an inequality between a microbial activity and soil organic matter mineralization. This usually happens during a stressful situation caused for example by presence of organic pollutants. According to metabolic quotient (Table 5), the best variants were once-a-year-mulched mixture and once- or twice-a-year-mulched legumes. A black fallow as well as a spontaneous fallow were characterized with the highest values of qCO_2. The variants mulched once were the plots where a more pronounced mineral carbon immobilization into microbial cells took place (high value of MBC) and so qCO_2 was the lowest one. Overall, mulched mixtures or legumes are the best variants for arable soil setting aside. AG period with the highest values of basal respiration and qCO_2 was typical for an unfinished stabilization after desiccation. This period would, with high probability, continue until the qCO_2 declines from 0.30 under 0.24.

Carbon of soil organic matter (C_{org}). Carbon of soil organic matter provides us with the basic information about the supply of humic and non-humic substances in soil, and thus about the potential energy sources for soil microbial associations. It is an extraordinarily stable criterion, which shows a year-to-year non-measurable variation on soils without human interference. Our values of C_{org}, determined by colorimetric method (Sims and Haby 1971), for set-aside luvic chernozem ranged between 1.33 and 2.21%. According to Vance et al. (1987), Robertson et al. (1993), Kiem and Kandeler (1997) and Sato and Seto (1999) the value of C_{org} in arable and temporary grassed soils was in a very wide interval (0.25–3.49%). We have determined 1.54 ± 0.27% at BD period, which is very close to the values of Růžek et al. (2006); the usual value for chernozems is 1.52 ± 0.28%. At AD period, a considerable amount of root mass remained in the soil profile of 0–200 mm. This root matter was, due to an extremely drought period (August–November 2003), very slowly mineralized and immobilized into microbial biomass. This fact affected several parameters including C_{org}, as its values increased up to 27%. Nevertheless, throughout the entire experiment period (BD, AD and AG) the differences in C_{org} among the eleven observed plots were highly significant. Our variant of once- or twice-a-year-mulched legumes is an alternative to the results of Mijangos et al. (2006), who used more than ten biological parameters and also confirmed the best situation on soils with organic fertilizing but without ploughing.

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