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The effect of management practice of montane meadows in the Bohemian Forest on selected soil biological and chemical properties

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Abstract
Selected properties of soil microbial community and some soil chemical characteristics were studied in relation to the management of montane grassland in the Bohemian Forest. The grassland had been regularly mown until 1997 when the field experiment was established. Three experimental treatments were investigated: mowing, mulching and abandonment. For the purpose of this study, the soils were sampled in 1999 and 2000. It was found that both mulching and abandonment led to decrease in both soil microbial biomass and overall microbial activity expressed as dehydrogenase activity, as compared to the control mown plot. In contrary, there was no clear effect of management practice on basal soil respiration and denitrifying enzyme activity. Study on soil nitrogen availability showed rather distinct pattern of ammonium and nitrate nitrogen content in the soils, as well as major differences in accumulation of the both nitrogen forms in soil profiles of the three variants. Laboratory experiment focused on the rate of cellulose mineralization in the soils under study revealed an increased decomposition of organic substrate in the mulched and abandoned plots in comparison with the mown variant. It was concluded that shift of grassland management from mowing to mulching and/or abandonment caused changes in soil microbial community and in nutrient cycling in a relatively short time-period.

Key words: soil, microbial community, grassland, mulching, mowing, nitrogen, organic matter

Introduction

Permanent semi-natural grassland is an important component of the Bohemian Forest landscape. In past decades, it was typically used for hay production as well as grazed. Nowadays, however, most of montane meadows are in unsatisfactory condition, which is related to political, social and economical changes in the region after 1989. Many new landowners and farmers have only limited resources to take care about all their land including the grassland. In general, this results in an inadequate management and some montane grassland sites are even abandoned. Thus there is a need for a management practice which (i) could replace the traditional mowing or grazing, being cheap, simple and available also in marginal regions, (ii) would reduce the intensity of grassland use, (iii) would be compatible with the general conservation strategy in the Bohemian Forest area. Mulching has been proposed as an alternative

management practice for montane grassland in recent years. However, although traditionally used in many agro-ecosystems (e.g. in orchards or vineyards, and in different arable cropping systems as well), mulching is a rather novel management practice in mountain areas. While in arable farming systems the mulch is used for soil and water conservation and although the beneficial effects of mulching in such systems are widely recognized (e.g. LAL 1994), there is only little experience with the impact of mulching on montane grassland. Mulching will presumably change the vegetation pattern, namely its species variety and diversity, and its productivity. Changes in accumulation of plant biomass and litter, together with other physico-chemical effects caused by mulching, could relatively soon lead to various (unknown) impacts on the soil microbial community and, consequently, would change the nutrient turnover, rates of important biological processes, etc., regardless of existing soil resilience. This study was thus designed to evaluate the possible effects of mulching on selected soil biological properties in a montane meadow. A mulched stand was compared with a mown grassland and in addition an untreated (abandoned) plot was studied. The study is a part of a large project focused on various aspects of different management practices of montane grasslands (see MAŠKOVÁ & al. 2001a).

MATERIALS AND METHODS

Study site and soil sampling

The experimental site is located at Hutská Hora Mt. near Horská Kvilda in the Bohemian Forest ca. 1150–1180 m a.s.l. In 1997 a field experiment was established focused on the effects of mulching on grassland vegetation and soil under the sward. The experimental treatments involve: (i) mulching (once a year, usually in mid July, abbreviated here as "M"), (ii) abandoned field ("A") and (iii) control plot ("C"), which is mown once a year (at the same dates as mulching is applied). For details about the site and experimental setup see MAŠKOVÁ & al. (2001a,b).

Soil was sampled from the experimental plots three times in 1999 (on May 11, July 7, and October 18) and 2000 (on May 18, July 17, and October 23) from the top 15 cm soil layer (the uppermost ca. 3–5 cm layer composed mostly of plant roots was excluded). Five composite samples (each of 3 subsamples; about 3 kg of soil) were collected at random from each treatment (i.e. mulching, mowing, abandonment) in polyethylene bags and placed into insulated boxes to avoid major temperature changes during transportation. The field-moist soils were passed through a 2-mm sieve into sealed bags and allowed to equilibrate at 4±2°C in darkness for 4–5 weeks. Prior to short-term biological analyses (DHA, DEA, see below) they were equilibrated at 22–25°C. For details on soil physico-chemical properties see KVÍTEK & al. (2001).

Analyses

Microbial biomass C and N – Carbon in microbial biomass (Cmic) was estimated using the $CHCl_3$ fumigation-extraction (VANCE & al. 1987). For estimation of nitrogen in microbial biomass (Nmic), the modified method of CABRERA & BEARE (1993) was used. Briefly, 10 g subsamples of moist soil were extracted with 40 ml 0.5-M K_2SO_4 for 30 min, filtered and filtrates were used for analyses of C and N. At the same time, 10 g subsamples of field moist soil were fumigated with ethanol-free $CHCl_3$ for 24 hours. After fumigation, $CHCl_3$ was purged from the soil and the subsamples were immediately extracted and filtered as those of unfumigated soil. Eight ml of sulphate extract was used for determination of total C content, 1 ml for analysing total N content. Carbon content was determined by dichromate digestion method. Con-

version factor (k_{EC}) used for Cmic calculation was 0.38. Total N was determined by an alkaline persulfate oxidation method. N was oxidized by peroxydisulfate ($K_2S_2O_8$) to NO_3^- at elevated temperature in an alkaline environment and NO_3^- was analysed by flow injection analyser (Tecator FIAStar 5020). Conversion factor (k_{EN}) used for Nmic calculation was 0.45. The analyses were carried out using 4 replicates.

Basal respiration – Basal respiration was measured using the absorption method of JÄGGI (1976). 200 g moist soil was incubated in a closed glass bottle (0.75 l) with 10 ml 1-M NaOH for 10 days at 20°C. The amount of CO_2 trapped in the alkali was estimated by titration with 0.5-M HCl on the indicator phenolphthalein. The analyses were carried out using 5 replicates.

Cellulose mineralization and cumulative soil respiration – A weighted cellulose filter paper (ca 0.5 g, ash-free) in a nylon bag was buried in 200 g of moist soil in a closed glass bottle (0.75 l) and incubated at 20°C. Soil respiration was measured as carbon dioxide evolution at regular intervals six times during the 40-d incubation using the absorption method described above. The results obtained were used to calculate cumulative soil respiration. After 40 d of incubation, the filter paper was taken out, dried at 105°C and weighed. The amount of decomposed cellulose was calculated from the differences in the weight of filter at the beginning and at the end of incubation. The analyses were carried out using 5 replicates.

Dehydrogenase activity – Dehydrogenase activity (DHA) was determined using the method of CASIDA & al. (1964). Soil samples (3 g) were mixed with 0.04 g $CaCO_3$, 1 ml of 3% aqueous triphenyl-tetrazolium chloride (TTC) solution and 2.5 ml of distilled water in test tubes. The tubes were sealed, shaken and incubated at 37°C for 24 hours. TTC-formazan was extracted from the soil suspension with methanol, filtered and made up to 50 ml with additional methanol. The absorbance at 485 nm (1 cm path length) of the extracts was measured by spectrophotometry (Jenway 6105) using methanol as a blank. The analyses were carried out using 4 replicates.

Denitrifying enzyme activity – Denitrifying enzyme activity (DEA) was determined using an anaerobic slurry technique of SMITH & TIEDJE (1979). Slurries were made by adding 25 ml of a solution containing 1 mM glucose, 1 mM KNO_3 and 1 $g.l^{-1}$ chloramphenicol to 25 g soil in 120 ml serum bottles. Bottles were sealed with butyl rubber stoppers, evacuated and flushed four times (for 2 minutes each) with oxygen-free helium. Ten ml of acetylene was added to each bottle and the internal pressure was equilibrated to atmospheric pressure using a water valve. The soil slurries were shaken on an end-over-end shaker at 25°C. 30 and 60 minutes after acetylene addition, 1 ml samples of head-space gas were taken from each bottle and N_2O was determined by gas chromatography (HP 5890A equipped with an electron capture detector and 3.2 mm × 3.5 m 80–100 mesh Porapak Q stainless-steel column); allowance was made for dissolved N_2O (TIEDJE 1982). The analyses were carried out using 4 replicates.

Soil nitrogen availability – Ion exchange resin (IER) bag method was used for the estimation of soil N availability (Nav) (BINKLEY & MATSON 1983, BINKLEY 1984). In contrast to all other measures, the Nav was determined *in situ*. Resin bags were prepared by placing ion exchange resins (CER, cation exchange resin No. Purolite C100E, and AER, anion exchange resin No. Purolite A520E) in cylindrical stockings (0.9 cm diameter by 21.3 cm long), made of fine nylon mesh (grid size 42 µm) which were then stapled shut. The bags contained either 11.2 g moist weight cation exchange resin (13.7 ml, 5.4 g dry weight) or 9.3 g moist weight anion exchange resin (13.7 ml, 4.9 g dry weight). Exchange sites of IER were saturated with Cl^- and Na^+ ions. Eleven CER bags and eleven AER bags were randomly inserted into cylindrical holes (1.0 cm diameter) in each of the three experimental treatments. The holes were prepared by pushing a metal rode into a soil profile at a 45° angle to a soil depth of about 15 cm. In 2000, one set of bags was deployed for the first part of the growing season (from June 13 to September 14) and another set of bags for the following period (from September

14 to October 23). For the quantification of NH_4-N and NO_3-N trapped by the resins, the IER bags were allowed to dry at room temperature. The resin was then separated from the nylon stockings into two different parts corresponding to soil depths of 0–5 cm and 5–15 cm, respectively. Absorbed NH_4-N and NO_3-N were eluted from IER using 100 ml 1.7-M NaCl and determined by distillation and titration method (PEOPLES & al. 1989). Content of NH_4-N and NO_3-N extractable with 2-M KCl in soil layers of 0–5 cm and 5–15 cm was determined by the same method (PEOPLES & al. 1989) in soils sampled at the beginning and at the end of each period of the IER bags exposure.

Unless otherwise stated, results are expressed per gram or kilogram of dry soil. The differences between means were evaluated by ANOVA/MANOVA followed by Tukey test, using STATISTICA software (version 5.1 for Windows).

Results and Discussion

The majority of results on the soil microbial community obtained in a 2-year study is summarised in Table 1. The table shows results on carbon and nitrogen in soil microbial biomass (Cmic and Nmic, respectively), soil respiration determined as carbon dioxide evolution (Resp), activity of dehydrogenase enzymes (DHA) and activity of denitrifying enzymes (DEA) in three experimental treatments.

The microbial biomass was relatively high in the control soil (C, mown sward) compared to both abandoned (A) and mulched (M) variants, which is documented by a higher Cmic on many sampling dates as well as higher mean values for both 1999 and 2000 seasons. In addition, Nmic determined in 2000 was also higher in the control soil. The average ratio between Nmic and Cmic was slightly different in different variants: while it was only 0.22 in control soil of the mown sward, in abandoned and mulched variants it was 0.23 and 0.27, respectively. We suggest that an increased N/C ratio in the mulched variant indicates either an increased proportion of bacteria in the microbial community or its enhanced activity. The former idea is supported by data on dehydrogenase activity in soils under study. As shown in Table 1, both abandonment and mulching led to decreases in the DHA, which represents a sort of overall microbial activity in the soil. The increased N/C ratio in the soil under mulching could then indicate rather changes in the community structure than an enhanced microbial activity in the mulched variant. These data suggest that shifts in the grassland management leading to a greater accumulation of plant biomass and litter, i.e. both mulching and/or abandonment, had a relatively fast (about 4 years after the onset of the different management) impact on soil microbial community. The effects of management practice on the soil microbial community may be direct or probably indirect, also being related to changes in the availability of nutrients or rates of nutrient turnover in the ecosystem.

In contrary to the above results on Cmic, Nmic and DHA, there was no clear effect of the management practice on basal soil respiration (Resp) and on denitrifying enzyme activity (DEA). The Resp, however, represents total carbon dioxide evolution from the soil during incubation under laboratory conditions and involves both microbial and invertebrate respiration; in addition evolution of CO_2 from fine roots present in the soil sample cannot be omitted. The relative portions of the three potential CO_2 sources are unknown. The Resp thus may not be sensitive enough to reflect changes in the soil microbial community. On the other hand, DEA is much more specific measure, which is probably related to the size of the community of denitrifying bacteria (SMITH & TIEDJE 1979) and represents current enzymatic activity in the soil (see ŠIMEK & al. 2000). Similar DEA in all variants suggests that management practice did not influence the denitrifying part of the soil microbial community (there were indications of a lower DEA in mulched variant in comparison to other two variants in several cases, but

Table 1. – Selected characteristics of soil microbial community in three experimental treatments (C = control, i.e. mowing, A = abandonment, M = mulching). Means of 5 samples (unless otherwise stated) and standard deviations in parentheses. Different letters indicate significant differences ($P < 0.05$) between the treatments within individual sampling dates.

Characteristic[1] (units)	Treatment	1999				2000				mean 1999–2000[3]	% (mean 1999–2000 C=100)
		May 17	July 7	October 18	mean 1999[2]	May 18	July 17	October 23	mean 2000[2]		
Cmic (mg C.g^{-1})	C	1091 (183) a	1260 (147) a	1026 (111) a	**1126 (121)**	948 (122) a	974 (115) a	1114 (92) a	**1012 (89)**	**1069 (114)**	100.0
	A	887 (135) ab	936 (137) b	843 (144) a	**889 (46)**	934 (187) a	891 (71) a	805 (241) b	**877 (66)**	**883 (51)**	82.6
	M	734 (75) b	946 (77) b	776 (91) a	**818 (112)**	677 (89) a	852 (119) a	662 (91) b	**730 (106)**	**774 (109)**	72.4
Nmic (mg N.g^{-1})	C	nd	nd	nd	-	250 (20) a	239 (20) a	171 (19) a	**220 (43)**	-	-
	A	nd	nd	nd	-	229 (13) a	235 (20) a	133 (14) b	**199 (57)**	-	-
	M	nd	nd	nd	-	224 (21) a	232 (25) a	126 (13) b	**194 (59)**	-	-
Resp (mg C.g^{-1}.h^{-1})	C	1.38 (0.12) a	2.61 (0.18) a	1.00 (0.15) a	**1.66 (0.84)**	0.59 (0.06) a	0.81 (0.03) a	0.79 (0.05) a	**0.73 (0.12)**	**1.20 (0.74)**	100.0
	A	1.40 (0.12) ab	2.71 (0.32) a	1.05 (0.11) a	**1.72 (0.88)**	0.57 (0.08) a	0.99 (0.09) a	0.77 (0.03) a	**0.78 (0.21)**	**1.25 (0.77)**	104.2
	M	1.46 (0.11) b	2.43 (0.22) a	1.06 (0.14) a	**1.65 (0.70)**	0.58 (0.05) a	0.93 (0.10) a	0.77 (0.12) a	**0.76 (0.18)**	**1.21 (0.67)**	100.8
DHA (mg TPF.g^{-1}.h^{-1})	C	nd	20.8 (4.3) a	17.8 (1.7) a	**19.3 (2.1)**	28.9 (2.4) a	27.4 (4.0) a	25.7 (1.1) a	**27.3 (1.6)**	**24.1 (4.7)**	100.0
	A	nd	14.5 (1.8) a	14.4 (1.1) a	**14.5 (0.1)**	16.9 (2.0) b	25.1 (1.7) a	21.7 (4.7) a	**21.2 (4.2)**	**18.5 (4.7)**	76.8
	M	nd	15.5 (1.8) a	20.6 (3.5) a	**18.1 (3.6)**	23.4 (4.8) a	21.8 (3.3) a	21.3 (2.5) a	**22.2 (1.1)**	**20.5 (3.0)**	85.1
DEA (mg N.g^{-1}.h^{-1})	C	454 (158) a	789 (120) a	589 (64) a	**611 (169)**	762 (88) a	619 (98) a	532 (160) a	**638 (116)**	**624 (130)**	100.0
	A	510 (198) a	698 (157) a	574 (130) a	**594 (96)**	661 (80) a	725 (60) a	599 (137) a	**662 (63)**	**628 (81)**	100.6
	M	464 (126) a	538 (200) a	516 (142) a	**506 (38)**	727 (145) a	633 (110) a	445 (129) a	**602 (144)**	**554 (108)**	88.9

[1]Cmic = carbon in microbial biomass, Nmic = nitrogen in microbial biomass, Resp = basal soil respiration, DHA = dehydrogenase activity, DEA = denitrifying enzyme activity

[2] means and standard deviations in parentheses, n = 3, for DHA in 1999 n = 2

[3] means and standard deviations in parentheses, n = 6, for DHA n = 5; nd = not determined

due to relatively large variability and inconsistent results during the seasons it is difficult to distinguish differences in DEA between the variants).

Studies on soil nitrogen availability were carried out in 2000 only. Actual amounts of soil mineral nitrogen in the three experimental plots and on all sampling dates and in two soil layers were quite low (Table 2). The greatest difference between the actual amounts of ammonium nitrogen was recorded in the abandoned plot in the 0–5 cm soil layer on September 14 and October 23 (13.30 and 1.31 mg $N.kg^{-1}$, respectively). In an early summer (June 13) the actual amount of nitrate nitrogen in both layers of the control plot was below the detection limit, while on the last sampling date (October 23) the highest amounts of NO_3-N were found in the same plot in both layers (3.25 and 2.34 mg $N.kg^{-1}$ in the 0–5 cm and 5–15 cm, respectively) (Table 2).

Results on ammonium and nitrate nitrogen accumulation in ion exchange resins (IER) buried in the soils are displayed in Fig. 1. Although the sum of both nitrogen forms trapped during the whole experimental season (from June 13 to October 23) was rather similar in all plots (summary data not shown), clear differences between the plots were detected in the individual periods of study. In the first period (from June 13 to September 14), both the amount and the proportion between the two N-forms accumulated in the IER were quite different in different plots, indicating differences in nitrogen transformation processes and nitrogen cycling (Fig. 1a). There was no nitrate nitrogen accumulation in the mulched plot and neither nitrate nor ammonium nitrogen in deeper soil layer of the control mown plot. On the contrary, large amounts of ammonium nitrogen and ammonium nitrogen plus nitrate nitrogen were present in soils of the mulched and abandoned plots, respectively (Fig. 1a).

Low total amount of nitrogen accumulated in the soil under the mown sward in the first experimental period (which involves mowing, too) may indicate temporary insufficient N-availability as a possible consequence of (i) high microbial immobilisation of nitrogen due to changed physical conditions just after mowing, which is followed by an increased plant demand for nitrogen necessary for re-growth, and (ii) the limited supply of nutrients (nutrients contained in plant biomass are removed each year after mowing). On the other hand, if the vegetation is mulched and left to decompose on the site, nitrogen released in the form of nitrate is immediately consumed (as indicated by the absence of nitrate in the IER). But in general, there is enough nitrogen in the system, though in the form of ammonium (corresponding to a high amount of ammonia in the IER). Cycling of nitrogen in the abandoned plot is different, as indicated by the accumulation of both nitrate and ammonium (see Fig. 1a). Later in the season, however, completely different amounts of nitrogen were accumulated in the IER in the individual soils and the proportion of the two forms was also different (Fig. 1b).

Table 2. – Contents of ammonium and nitrate nitrogen in the soils from control (mown), mulched and abandoned plots (mg $N.kg^{-1}$). Medians from 5 replicates are given.

Date	Soil layer (cm)	Control		Mulched		Abandoned	
		ammonium N	nitrate N	ammonium N	nitrate N	ammonium N	nitrate N
June 13	0–5	7.18	0.00	3.67	0.61	6.58	0.52
	5–15	2.38	0.00	2.29	1.13	1.96	1.55
September 14	0–5	6.63	1.79	7.82	1.06	13.30	1.18
	5–15	2.80	1.22	2.60	0.77	3.63	0.54
October 23	0–5	4.89	3.25	2.59	0.19	1.31	0.17
	5–15	3.95	2.34	1.42	0.00	1.04	0.20

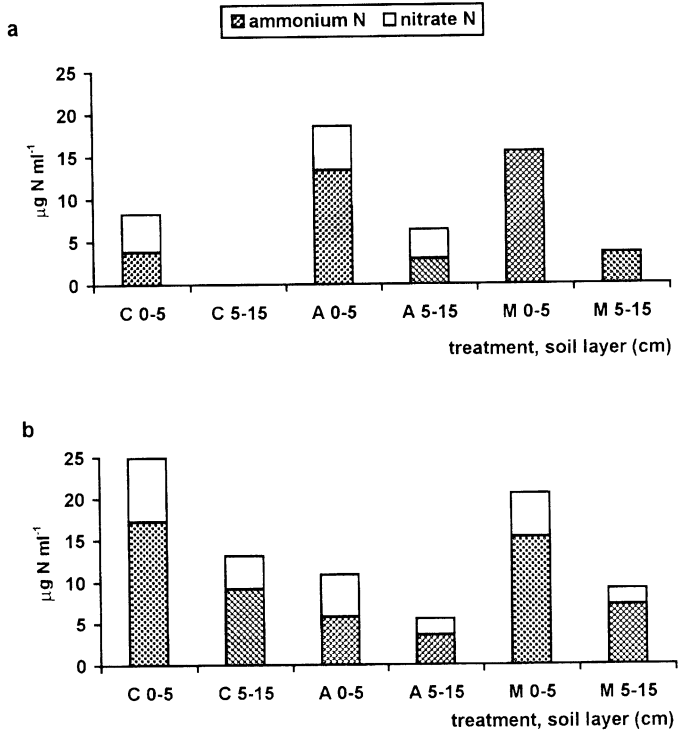

Fig. 1. – Accumulation of ammonium and nitrate nitrogen in ion exchange resins buried in the soils in control (C), abandoned (A) and mulched (M) plots from June 13 to September 14 (a) and from September 14 to October 23 (b). Data for two soil layers, 0–5 and 5–15 cm, respectively, are given and represent medians from 11 replicates.

All these data on nitrogen accumulation in the soil affected by grassland management clearly suggest that substantial changes in an internal (plant–microbes–soil) and presumably also an external (plant–microbes–soil–atmosphere) nitrogen cycling occur if different management practices are applied, despite the fact that the information available does not facilitate their detailed description and thus their understanding. In addition to the above circumstances, N loss via nitrate leaching after mowing (and mulching as well) later in the season cannot be omitted. This could be probably the combined result of the small demand for N in the grass-

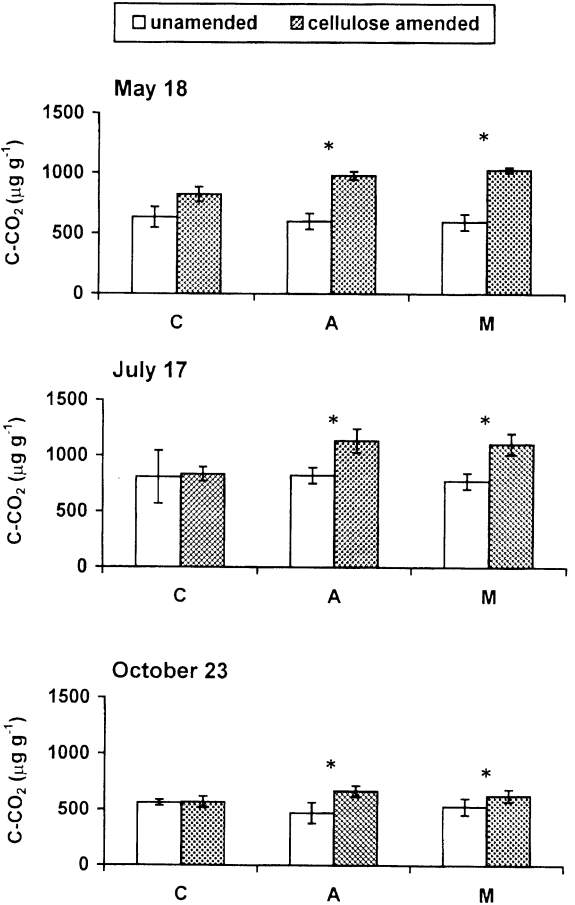

Fig. 2. – Respiration (cumulative carbon dioxide evolution during 40-d incubation) of soils from control (C), abandoned (A) and mulched (M) treatments, sampled three times in 2000. Mean data ± standard deviations of respiration of unamended and cellulose-amended soils are given (n = 5). Asterisks indicate significant differences ($P < 0.05$) between the unamended and amended soils.

land just after mowing or mulching and, presumably, high rates of microbial root decay and root exudation after cutting off the aboveground plant parts, as documented by GLOSER & al. (2000).

In a separate laboratory experiment, the rate of cellulose mineralization in soils from the three experimental treatments sampled in 2000 was evaluated and related to soil respiration. Cellulose addition to the soils under study enhanced significantly carbon dioxide evolution from A (abandoned) and M (mulched) soils only (Fig. 2). In contrary, there was only a minor response of the control (C) soil to the cellulose amendment. This could be explained by a general limitation of microbial community in the C soil due to lack of available nutrients (N, P, and others), or, less probably, by a higher content of undecomposed organic residues and litter in the C soil (indicated by higher amounts of extractable carbon found in this soil during microbial biomass carbon determination – data not shown). If the level of organic carbon in the soil is high enough, the small amendment of additional organic substrate might not be accompanied by an increase of carbon dioxide evolution from such soil. The content of plant residues and litter in the studied soils was, however, not checked. The rate of cellulose mineralization/decomposition was relatively low in the C soil (data not shown), corresponding thus to the negligible response to the cellulose amendment mentioned above. The relatively highest rate of cellulose mineralization was found in the A soil, but the differences between the M and A soils were not significant.

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