

Effects of liming on the microbial biomass and its activities in soils long-term contaminated by toxic elements

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ABSTRACT

The effects of liming by CaO and CaCO₃ on soil microbial characteristics were studied during laboratory incubation of long-term contaminated arable and grassland soils from the vicinity of lead smelter near Příbram (Czech Republic). The CaO treatment showed significant negative effects on soil microbial biomass C and its respiratory activity in both studied soils, despite the fact that microbial biomass C in the grassland soil increased sharply during the first day of incubation. The metabolic quotient (qCO₂) in soils amended by CaO showed greater values than the control from the second day of incubation, indicating a possible stress of soil microbial pool. The vulnerability of organic matter to CaO could be indicated by the availability of K₂SO₄-extractable carbon that increased sharply, particularly at the beginning of the experiment. The amendment of soils by CaCO₃ moderately increased the soil microbial biomass. The respiratory activity and qCO₂ increased sharply during the first day of incubation, however it is not possible to ascribe them only to microbial activities, but also to CaCO₃ decomposition in hydrogen carbonates, water and CO₂. The pH values increased more sharply under CaO treatment in comparison to CaCO₃ treatment. The improvement of soil pH by CaCO₃ could be therefore more convenient for soil microbial communities.

Keywords: liming; soil microbial biomass; respiratory activity; metabolic quotient qCO₂; heavy metals

Soil contamination by toxic elements is one of the major environmental problems because of the large inputs of toxic elements over the past centuries. The large adverse effects of heavy metals emissions from smelters on surrounding ecosystems and the microorganisms were observed from 1960–1970. Until now a considerable body of information has been accumulated on the effects of heavy metals on soil microorganisms and microbially-mediated processes from laboratory studies and field experiments (Giller et al. 1998). The significant negative effects on microbial biomass and its metabolic activities are described in many studies (i.e. Brookes 1995, Leita et al. 1995, Nannipieri et al. 1997), however, there is an enormous disparity between studies, as to which metal concentrations are toxic (Bååth 1989). For instance, Mühlbachová and Šimon (2003) showed that the activity of the microbial pool could differ among the studied contaminated areas and that in long-

term contaminated soils it could not be necessarily lower in the presence of toxic elements.

Together with the understanding of the effects of toxic elements on natural ecosystems, the soil remediation practices have been developed in the past years. Among the techniques available for soil remediation the metal immobilization plays a significant role. Liming is a common practice for reducing the mobility of heavy metals (Kuntze et al. 1984) because it does not only support the plant nutrition by calcium, but it improves the soil properties and is often used to decrease the heavy metals mobility.

High acidity inhibits the growth of soil bacteria in favour of more resistant fungi. In contrast, liming which increases the soil pH usually improves the bacteria growth. The recovery of soil bacteria after heavy metals amendment was faster when soil pH, which had decreased due to the metal addition, was restored by liming (Rajapaksha et

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al. 2004). The increase of soil pH by liming is thus usually favourable for the microbial growth and it was found that microbial biomass increased already after two years after liming in comparison to unlimed control (Bezdicsek et al. 2003). Moreover, liming significantly increased total soil protein and these results were related to similar but less pronounced changes in microbial biomass in Cd contaminated and limed control soils (Singleton et al. 2003). In addition, the growth of microbial biomass and the growth of bacteria as the response to increased pH in limed soils were commonly found (i.e. Geissen et al. 1998, Chagnon et al. 2001, Lorenz et al. 2001, Reichardt et al. 2001, Oyanagi et al. 2004). Waschkies and Huttl (1999) however reported that the concentration of microbial biomass increased almost linearly with the pH range between 3.9 and 5.3 and no significant change in microbial biomass was detected with a further increase of pH above 5.3. On the other hand, in a study using $\text{Ca}(\text{OH})_2$ the numbers of colony-forming units (CFU) and the biomass of fungi decreased (Weyman-Kaczmarkowa and Pedziwilk 2000) indicating that the microbial biomass growth and activities could be inhibited by such types of liming. Furthermore, Groffmann (1999) did not observe any effect on biomass C after liming with Ca-Mg acetate or Ca carbonate, probably because the carbon addition stimulated microbial growth and activity, but there was no increase in microbial biomass due to the predation of the new biomass by soil fauna.

The aim of this research was to evaluate whether different forms of liming can affect the soil microbial biomass C and its activities in soils contaminated with toxic elements.

MATERIAL AND METHODS

The area about 60 km SW from Prague (Czech Republic) with long-term contaminated soils from the vicinity of the Příbram smelter was used in the experiment. The arable (P1) and grassland (P2) soil chosen for the experiment were typical

Cambisols according to the FAO classification (Mühlbachová and Šimon 2003, Šichorová et al. 2004, Mühlbachová et al. 2005). The Příbram smelter has been in operation since 1786. Metal mining was ceased in 1972, but a secondary lead smelter has still been running. Since 1982 a 98% efficient dust separator and a 160 m stack have been in use (Kalac et al. 1991, Riuwerts and Farago 1996).

Soils were sampled in the autumn 2003 (layer 0–200 mm) making 6 samplings in the circle of 2 m from each sampling site; soil characteristics are shown in Table 1.

Seven days prior to the experiment, three replications of each treatment (1 kg of soil on an oven dry basis) in plastic jars were placed into 3 litre plastic containers, tightly covered with fitting lids, and they were conditioned at 28°C. The soils were preincubated at the 40% water holding capacity (WHC) with the jar of 25 ml 1M NaOH to take up the CO_2 -evolved and the distilled water at the bottom of the container. At the beginning of the experiment each soil replication was mixed with 3 g CaO/kg soil or 5.36 g CaCO_3/kg soil in order to obtain the same Ca concentrations for both amendments. The treated and control soils were adjusted to the 50% of its WHC. Thereafter, the amended soils were incubated under the above described conditions. The containers were aerated daily to ensure a sufficient oxygen supply. The soils were regularly adjusted to 50% WHC during the 1st year of incubation, a jar with 25 ml 1M NaOH in the containers was replaced weekly and the distilled water in one month intervals. The soils without lime amendment served as a control and they were conditioned as the treated ones for 28 days in the first part of the experiment; the last measure was performed after 365 days in order to compare the fresh lime application and the long-term effects.

The respiratory activity was determined by separate incubation of 100 g of soil weighed in three replicates for each treatment and incubated in 1 litre tightly closed plastic containers containing 5 ml 1N NaOH to determine the CO_2 -evolved. The

Table 1. Basic characteristics of soils from the Příbram area (pH, C_{org} , CEC, available Ca, K, Mg, P and toxic elements concentrations)

Soil	pH (H_2O)	Soil texture	C_{org} (%)	CEC (mmol/kg)	Ca	K	Mg	P	As	Cd	Cu	Pb	Zn
					mg/kg								
P1	6.50	loamy	1.53	171	2703	91.9	91.9	33.1	92.3	4.06	35.2	1138	255
P2	6.48	loamy	2.31	189	3214	41.9	83.5	22.5	127	9.01	34.3	2359	288

microbial biomass content, respiratory activity, pH and available toxic elements fractions were determined at days 0, 1, 2, 7, 14, 28 and 365 of the incubation.

The measurements of the soil microbial biomass C (B_c) were performed using the fumigation-extraction method (F.E.) according to Vance et al. (1987) procedure. The microbial biomass C was calculated from the relationship: $B_c = 2.64 E_c$, where E_c is the difference between organic C extracted from the fumigated and non-fumigated treatments, both expressed as $\mu\text{g C/g}$ oven dry soil.

K_2SO_4 -extractable carbon was determined by shaking of 20 g soil samples (an oven dry basis) for 30 minutes on an overhead shaker with 80 ml of 0.5M K_2SO_4 solution. The extraction was performed according to the determination of non-fumigated C described by Vance et al. (1987). Filtered extracts were determined on carbon content, similarly as microbial biomass measurements, by digestion with mixture of H_2SO_4 and H_3PO_4 and with $\text{K}_2\text{Cr}_2\text{O}_7$ and titration of excess dichromate with $(\text{NH}_4)_2\text{Fe}(\text{SO}_4)_2 \cdot 6 \text{H}_2\text{O}$.

The CO_2 C evolved was determined as an amount of organic C released as CO_2 after absorption in NaOH and precipitation with BaCl_2 and was analysed by titration with standard HCl on the automatic titrator Titrino 716. The metabolic quotient ($q\text{CO}_2$) was calculated according to Anderson and Domsch (1990) equation:

$$q\text{CO}_2 = \mu\text{g CO}_2\text{-C}/\mu\text{g CBc/h}$$

Values of soil pH were determined by combined glass electrode on the pH meter in the solution soil/water (1/2.5 w/v) after 1 h shaking on the overhead shaker.

RESULTS AND DISCUSSION

The CaO and CaCO_3 treatments had different effects on the microbial biomass C dynamics and the divers effects were observed also between arable and grassland soil (Figure 1). At the beginning of the experiment the CaO amendment decreased sharply the microbial biomass C in the arable soil (P1) from 171.4 $\mu\text{g C/g}$ soil to 91.6 $\mu\text{g C/g}$ soil and its subsequent increase did not reach the control and CaCO_3 values even one year after the CaO treatment (Table 2). The microbial biomass C in the grassland soil (P2) increased after CaO amendment from 333.4 $\mu\text{g C/g}$ soil to 596.2 $\mu\text{g C/g}$ soil. However, since the second day of incubation it remained, similarly to the CaO treated soil P1, lower than control and CaCO_3 treatment. The microbial biomass C decreased during the year of incubation in all studied treatments, but at the end of experiment, the largest microbial biomass C was found in CaO amended grassland soil. The addition of CaCO_3 in a one-year interval did not have any significant effects on microbial biomass development and was not significantly different from the control in both studied soils (Table 2).

The obtained results showed that the microbial biomass C was negatively affected by the CaO application already from the first day of the experiment in the arable soil (P1) and from the second day in the soil P2 (Figure 1). It is possible to suppose that the microbial biomass C in the grassland soil P2 increased under CaO treatment during the first day of incubation due to a greater amount of easily available carbon, which was possible to observe in both examined soils (Figure 2). The net decrease of the microbial biomass C as the response to CaO treatment during the incuba-

Table 2. The microbial biomass, K_2SO_4 -extractable C, respiratory activity, $q\text{CO}_2$, pH after 365 days of the incubation

Soil	Microbial biomass ($\mu\text{g C/g}$ soil)		K_2SO_4 -extractable C ($\mu\text{g C/g}$ soil)		Respiratory activity ($\mu\text{g C/g}$ soil/h)		$q\text{CO}_2$ ($\mu\text{g C}/\mu\text{g B}_c/\text{h}$)		pH (H_2O)	
	<i>x</i>	<i>s</i>	<i>x</i>	<i>s</i>	<i>x</i>	<i>s</i>	<i>x</i>	<i>s</i>	<i>x</i>	<i>s</i>
P1 – control	110.84	2.66	30.96	1.23	0.1636	0.0061	0.0015	0.0001	5.66	0.01
P1 – CaO	104.27	3.99	75.79	4.33	0.2679	0.0099	0.0026	0.0002	7.76	0.04
P1 – CaCO_3	112.72	0.00	60.84	3.70	0.2137	0.0039	0.0019	0.0002	7.74	0.01
P2 – control	174.33	0.00	46.90	5.20	0.1031	0.0166	0.0006	0.0002	5.47	0.03
P2 – CaO	192.16	8.40	73.91	4.69	0.2096	0.0099	0.0011	0.0002	7.25	0.01
P2 – CaCO_3	176.31	8.40	78.42	3.75	0.2674	0.0062	0.0015	0.0002	7.49	0.01

x = the media, *s* = deviation standard

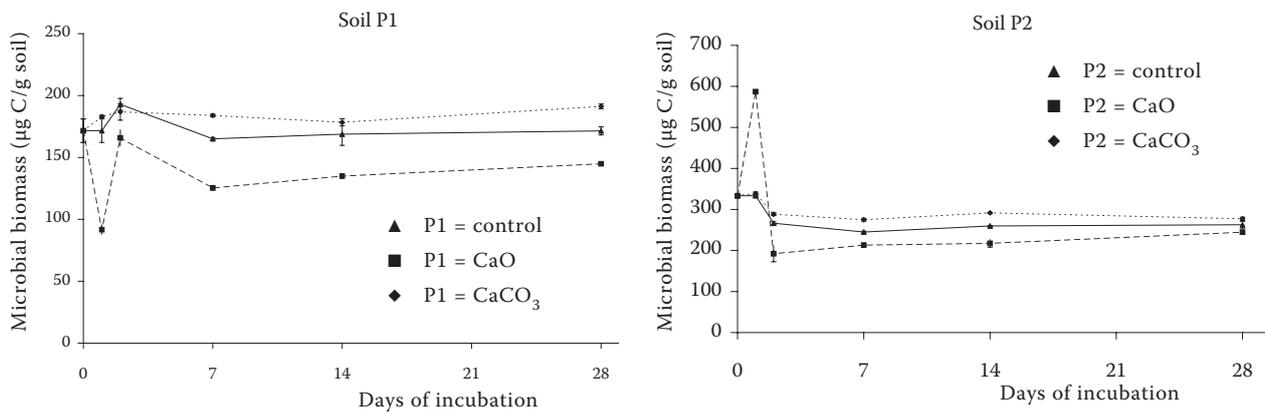


Figure 1. The microbial biomass dynamics in arable (P1) and grassland (P2) soil treated with CaO and CaCO₃ during laboratory incubation

tion suggests that the biomass C was disturbed and the soil organic matter rapidly mineralized due to the strong exothermic chemical reaction of soil water with CaO. On the other hand, the results obtained after one year of incubation did not confirm significantly lower microbial biomasses in comparison to control and the larger biomass C was found in the grassland soil (Table 2). In fact, Stenberg et al. (2000) found that liming with CaO increased microbial activities under long-term field experimental conditions.

The CaCO₃ application increased the microbial biomass C in both P1 and P2 from the 2nd and the 7th day of the incubation, respectively, indicating the positive effects of this type of liming on the microbial biomass C, although the increase represented only about 20 µg C/g soil. The application of limestone is largely used on uncontaminated soils to improve the soil properties and has usually positive effects on the microbial growth (Geissen

et al. 1998, Chagnon et al. 2001, Lorenz et al. 2001, Reichardt et al. 2001, Oyanagi et al. 2004).

After CaO amendment a significant increase of easily available C, expressed here as K₂SO₄-extractable carbon, was observed during the first day of incubation; up to 183.46 µg C/g for P1 soil and 174.34 µg C/g for P2 soil (Figure 2). Thereafter the K₂SO₄-extractable carbon decreased under CaO treatment throughout the incubation. K₂SO₄-extractable carbon in CaCO₃ amended soils increased regularly during the incubation from 30.77 µg C/g to 48.18 µg C/g at day 28 for the soil P1 and from 30.44 µg C/g to 68.82 µg C/g for the P2 soil at day 28. After one year the K₂SO₄-extractable C increased up to 60.68 µg C/g P1 soil and 70.95 µg C/g P2 soil.

The sharp increase of K₂SO₄-extractable carbon in CaO treated soils is probably linked to the exothermic reaction of CaO in the moist soil and subsequent disruption of the soil organic com-

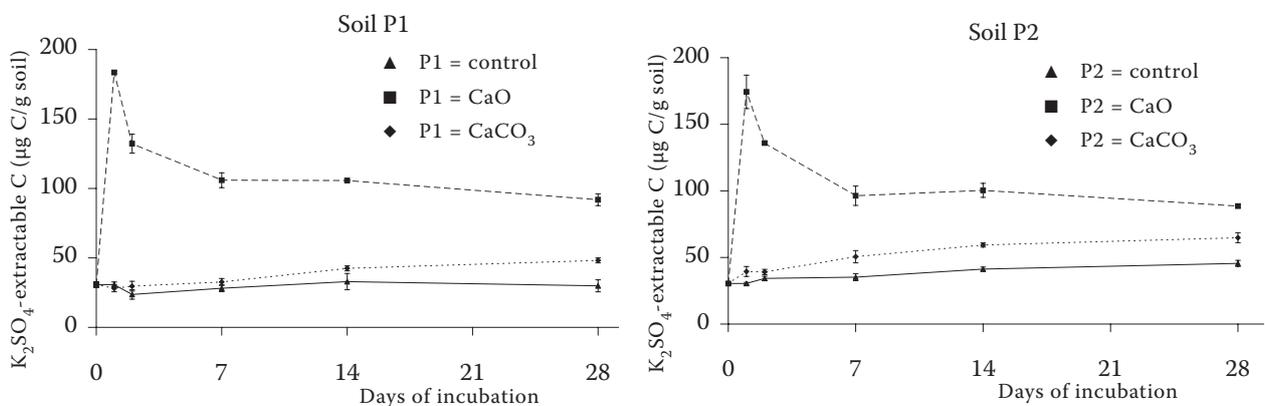


Figure 2. The K₂SO₄-extractable carbon dynamics in arable (P1) and grassland (P2) soil treated with CaO and CaCO₃ during laboratory incubation

Table 3. Correlation coefficients among selected soil characteristics

Soil	Treatment	Bc × RA	Bc × qCO ₂	Bc × pH	Bc × K ₂ SO ₄ C	RA × qCO ₂	RA × pH	RA × K ₂ SO ₄ C	QCO ₂ × K ₂ SO ₄	pH × K ₂ SO ₄ C	pH × qCO ₂
P1	control	0.399 ^{NS}	0.301 ^{NS}	0.905 ^{**}	-0.489 ^{NS}	0.988 ^{***}	0.627 ^{NS}	0.025 ^{NS}	0.104 ^{NS}	-0.198 ^{NS}	0.570 ^{NS}
	CaO	0.523 ^{NS}	0.251 ^{NS}	-0.635 ^{NS}	-0.633 ^{NS}	0.816 [*]	-0.468 ^{NS}	-0.410 ^{NS}	-0.206 ^{NS}	0.982 ^{***}	-0.200 ^{NS}
	CaCO ₃	0.352 ^{NS}	0.333 ^{NS}	-0.004 ^{NS}	-0.659 ^{NS}	0.903 ^{**}	-0.218 ^{NS}	-0.784 [*]	-0.693 ^{NS}	0.513 ^{NS}	-0.164 ^{NS}
P2	control	0.870 [*]	0.842 [*]	0.934 ^{**}	-0.839 [*]	0.985 ^{***}	0.751 ^{NS}	-0.943 ^{**}	-0.953 ^{***}	-0.791 [*]	0.780 [*]
	CaO	-0.101 ^{NS}	-0.407 ^{NS}	0.356 ^{NS}	0.488 ^{NS}	0.884 ^{**}	-0.323 ^{NS}	-0.338 ^{NS}	-0.165 ^{NS}	0.970 ^{***}	-0.096 ^{NS}
	CaCO ₃	0.754 [*]	0.560 ^{NS}	-0.469 ^{NS}	-0.830 [*]	0.910 ^{**}	-0.447 ^{NS}	-0.743 [*]	-0.731 ^{NS}	0.730 ^{NS}	-0.385 ^{NS}

Bc = microbial biomass C, RA = respiratory activity, K₂SO₄ C = K₂SO₄-extractable C, qCO₂ = metabolic quotient, pH = H₂O-extractable pH, statistical significance: NS – non significant, **P* ≤ 0.05, ***P* ≤ 0.01, ****P* ≤ 0.001

plexes. The direct effects of CaO treatment on carbon extractability also confirm highly significant relationships between pH and K₂SO₄-extractable carbon (Table 3). The liming usually improves soil microbial properties. Particularly the bacterial activity could be recovered after metal addition up to values similar to those of the control soil when soil pH, which had decreased due to metal addition, was restored to control values by liming (Rajapaksha et al. 2004). On the other hand, the form of used Ca amendment seems to be particularly important for carbon mineralization and microbial activities. Nevertheless, it is difficult to compare the incubation experiment and the liming with CaO in field trials with different tillage when CaO usually stabilized or improved soil microbial activities (Stenberg et al. 2000). The results after one year of incubation confirmed the decrease of K₂SO₄-extractable carbon in CaO treatments; relatively small differences were however obtained for microbial biomass. From the long-term view the microbial properties seemed not to be greatly affected by CaO, although the short-term incubation showed clearly negative effects of CaO on microbial biomass and K₂SO₄-extractable C availability. In addition, CaCO₃ application could mobilise soil organic matter as suggested Chander and Joergensen (2002). The increased contents of K₂SO₄ extractable C and increased microbial biomass C could confirm this opinion.

The CaO amendment decreased the respiratory activity in soils P1 and P2 during the first two days of the experiment (Figure 3). Thereafter the soil

respiratory activity increased over the control and it remained larger till the 28th day of experiment when no significant differences were observed.

The respiratory activity in CaCO₃ treatment increased significantly in both studied soils during the first day of incubation in comparison to control and CaO treatments. Thereafter, a progressive decrease in the soil respiratory activity was observed in both P1 and P2.

The metabolic quotient (qCO₂) (Figure 4) was found to be a sensitive indicator of soil microbial processes (Anderson and Domsch 1990). The highest qCO₂ was found in both CaCO₃ amended soils during the first day of incubation. In contrast, decreased qCO₂ in CaO treated variants during the first day (soil P2) and the second day (soil P1) of the incubation could be caused by the exothermic reaction of CaO in soils and by significantly decreased microbial biomass C, however the subsequent increase of qCO₂ was recorded.

Values of pH in both control soils decreased slowly during the incubation (Figure 5) but the different course of pH development was observed for CaO and CaCO₃ amendment. The pH in CaO treatment increased sharply up to 9.71 in the soil P1 and 8.9 in the soil P2. Afterwards the pH decreased slightly throughout the incubation, however it remained still higher than the CaCO₃ treatment till 28th day of the incubation and than the control. The CaCO₃ treatment increased pH continually up to the 14th day for the soil P1 and 28th day for the P2 soil. In comparison to the beginning of the incubation, the values of pH after one year were lower in all treatments. The CaCO₃ treat-

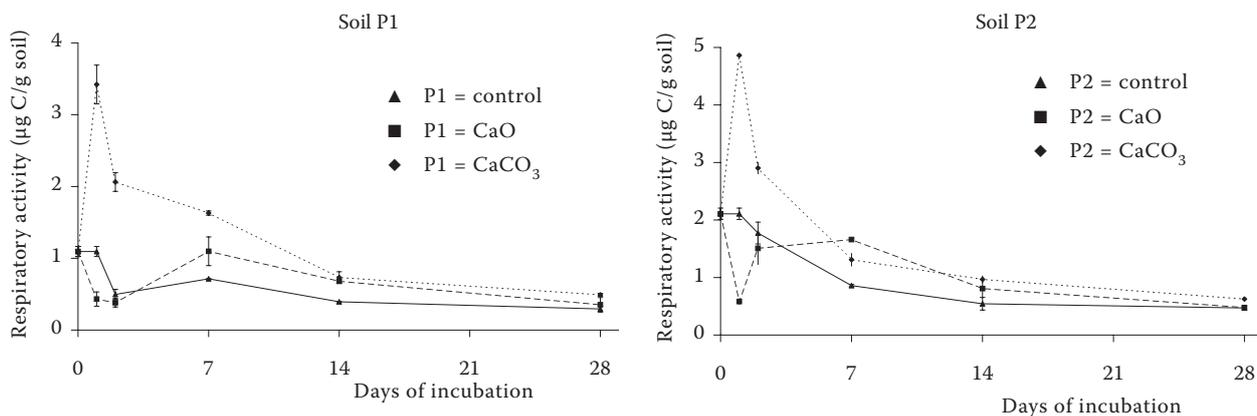


Figure 3. The respiratory activity in arable (P1) and grassland (P2) soil treated with CaO and CaCO₃ during laboratory incubation

ment showed higher pH in comparison to the CaO amendment and the lowest pH was observed in both control soils.

The relationships between microbial biomass C, K₂SO₄-extractable carbon and either respiratory activity or qCO₂ were significant only in control soils; in the case of K₂SO₄-extractable carbon they were significant also in the soil P2. In CaO or CaCO₃ amended soils no relationships were found (Table 3) suggesting that the changes in microbial activities after liming were so large that it is not possible to explain them by a simple correlation between these parameters. It is possible that the CaO and CaCO₃ amendments disequibrated the soil microenvironment. Great changes in microbial biomass C and in respiratory activity in CaO/CaCO₃ treatments, mainly at the beginning of the incubation, could explain the lack of relationships between these soil characteristics. On the other hand, the respiratory activity and

especially the qCO₂ usually reflect very well the microbial activity. An increase of the soil respiratory activity and qCO₂ under heavy metal stress was described by Brookes (1995) and Giller et al. (1998). It was generally explained as a result of a larger microbial activity and mineralization of soil organic matter (Brookes 1995) or stimulation of the microbial activity (Groffman 1999). On the other hand, Wardle and Ghani (1995) showed that this index could respond unpredictably and some disturbances such as fertilization or liming can either decrease or increase qCO₂. There is another possible explanation; the significant part of CO₂-evolved in CaCO₃ amended treatments did not derive only from the microbial respiratory activity or from the autolysed dead microbial cells and therefore it was probably not a result of a greater energy demand of microbial biomass C in contaminated environment (Brookes 1995, Giller et al. 1998) but of CO₂ evolution from applied CaCO₃.

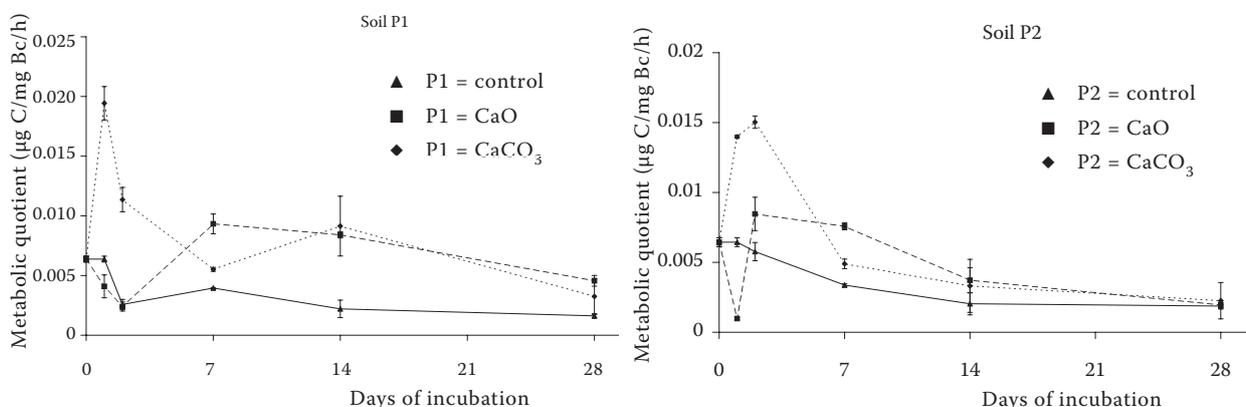


Figure 4. The metabolic quotient (qCO₂) in arable (P1) and grassland (P2) soil treated with CaO and CaCO₃ during laboratory incubation

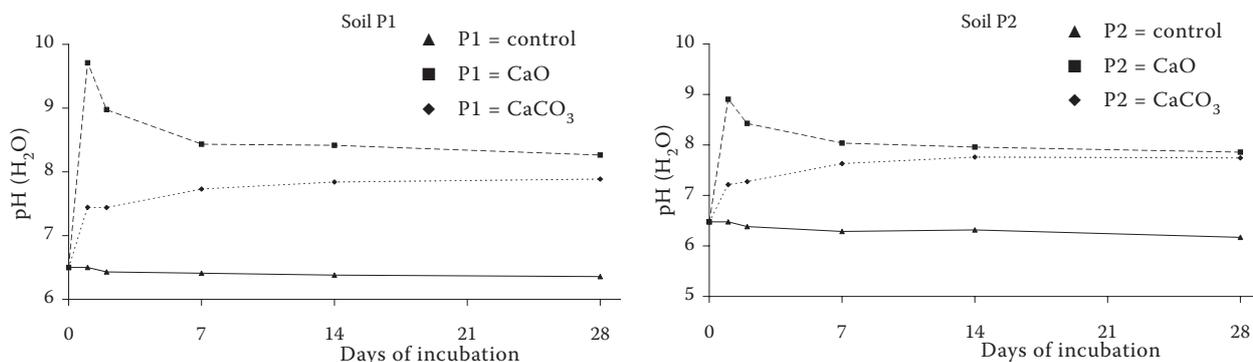


Figure 5. Values of pH (H_2O) in arable (P1) and grassland (P2) soil treated with CaO and $CaCO_3$ during laboratory incubation

No significant relationships were found between microbial biomass or respiratory activity and pH values in soils treated with CaO or $CaCO_3$, suggesting that the changes in microbial activities were too large to be explained by a simple correlation between these parameters. On the other hand, the significant relationships were found between K_2SO_4 -extractable carbon and CaO treatment in both arable and grassland soil confirming disturbing effects of the direct application of CaO on the soil organic matter (Table 3).

The form of liming was critical for the soil microbial activities. The CaO amendment decreased the soil microbial biomass; during the first days of incubation the respiratory activity and qCO_2 were also decreased. At last, the exothermic reaction of CaO caused rapid mineralization of the organic matter in soils. The $CaCO_3$ amendment had not so negative effects on the microbial biomass and its activities and from the microbial point of view it was more suitable liming agent for soils. However, possible dissociation of $CaCO_3$ molecule increased the respiratory activity and thus possible flux of CO_2 in the atmosphere.

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