Effect of liming on microbial biomass carbon of acidic arenosols in pot experiments

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ABSTRACT

In the paper we investigate the effect of liming on the microbial biomass carbon (MBC) in pot experiments during two vegetation periods. There was also another goal to get better understanding of the role of dissolved organic matter (DOM) and its quality on microbial processes. Pot experiments were carried out on two acidic soils. Liming material treatment was 0, 1, 2, 3 g $CaCO_3$/kg soil (corresponding with 0, 1.4, 2.8, 4.1 t $CaCO_3$/ha, respectively). On both soils, 3-3 soil samples were taken for two growing periods and the substrate-induced respiration (SIR), dissolved organic carbon and nitrogen (DOC and DON), and soil pH were determined from the soil samples. The SIR can be used to characterize the active biomass within the total microbial biomass. Liming was found to increase soil respiration and consequently MBC in the first year of the experiment, but at the maximum lime rate these values stagnated or declined in many cases on each soil. In the second year, the effects of treatments were much lower both on Kisvárda and on Nyírlugos soils. Under the given experimental conditions, when the DOC/DON ratio rose to above 30–40, disturbances appeared in N supplies to microorganisms. The N content of the easily mineralisable organic matter in the soil became so low that it inhibited the reproduction of the microorganisms.

Keywords: liming; acid soils; microbial biomass carbon; dissolved organic matter; dissolved organic carbon; dissolved organic nitrogen

The aim of liming to acid soils is to improve soil fertility. Adding liming materials to soils can change a number of soil parameters, resulting at brand new circumstances for soil microorganism. Liming effects on microbial biomass were investigated by numerous researchers, but mainly in forest, meadow or pasture areas. Based on the relevant literature, it is clear that soil pH increase after liming caused a significant MBC increase of soils investigated (Anderson 1998, Chagnon et al. 2001). Similar findings were obtained in experiments carried out in agricultural soils: the lime application increased the MBC and microbial activity of soils (Bezdicek et al. 2003, Soon and Arshad 2005).

Besides the favourable pH conditions, sufficient substrate is necessary for the microorganisms to increase the amount of microbial biomass. The more important fraction of soil organic matter, so called dissolved organic matter (DOM) fraction, plays a prominent role in microbial decay of organic matter, which was proved by investigations in which correlation was found between CO_2 release and DOM concentrations of soil (Marschner and Noble 2000). Moreover, concentration of DOM changed through liming itself, and in most cases the liming increased the DOM content (Filep et al. 2003). In addition to this, liming can changed the microbial community resulting in the decrease in MBC/MBN ratio, indicating that bacteria become predominant over fungi when soil acidity decreases (Badalucco et al. 1992).

In our study, we determined the substrate-induced respiration (SIR) of soils, which can be used to characterize the active biomass within the total microbial biomass. Besides this, the measure of SIR enables to calculate microbial biomass C as well. Our paper was made to obtain supplementary information about the liming effects on microbial activity on sand soils, to get data on the connection of liming, microbial activity and dissolved organic matter.

MATERIALS AND METHODS

The used soils were two Luvic Arenosols (FAO-ISRIC-ISSS, 1988) from Kisvárda and Nyírlugos.

Table 1. Physical and chemical properties of soils used in pot experiments

	Kisvárda 47°41'N 22°02'E	Nyírlugos 47°42'N 22°01'E
pH ($CaCl_2$)	4.4	4.2
Hydrolytic acidity (y_1)	12.6	8.1
Sand (%)	86.7	84.5
Silt (%)	6.6	5.2
Clay (%)	6.7	10.3
K_A*	31	25
CEC (cmol/kg)	2.7	8.6
Organic C (%)	0.41	0.35
C/N	7.8	8.9

*upper limit of plasticity

Table 2. Average temperature (°C) in growing periods of investigation

	2000	2001	2002	2003
May	17.6	17.6	17.2	18.7
June	20.1	18.1	18.6	20.8
July	20.2	21.8	22.0	21.2

The samples were taken from the 0–20 cm layer, were air-dried and passed through a 2-mm sieve. The selected physical and chemical properties of soils are shown in Table 1.

Pot experiment. A pot experiment was set up at the University of Debrecen, Hungary. 11 kg of soil was dispensed into each pot. Water content was set to 75% of the water capacity. Water loss via evapotranspiration was determined by weighing and was replaced with deionised water. Table 2 contains average temperature data for growing season of the experiments.

The experimental design was randomised complete block with 4 treatments and 18 replicates. The treatments applied are shown in Table 3. The liming materials were mixed thoroughly with the soil. Treatments were performed with the same amount 90.9 mg/kg soil of N, P and K (introduced in a mixture of $NH_4H_2PO_4$, NH_4NO_3 and KCl).

We used the Kisvárda soil from 2000 till 2001, and the Nyírlugos soil from 2002 till 2003. 40 oat seeds (*Avena sativa* L.) were sown into each pot on 18th April 2000 and 24th April 2002, respectively. Treatment effects were observed over two vegetation periods, taking three soil samples during each vegetation period.

In the first years of experiments, i.e. 2000 and 2002, the sampling times were: 18th May, 14th June, 21st July; and 23rd May, 20th June, 18th July, respectively.

After harvesting, the remainder pots were left till the next season in 2000 and 2002. In 2001 and 2003 (2nd years of experiments), the procedure was almost the same as in 2000 and 2002, but only NPK solutions were added to the soils, with no calcite treatments. Oat was sown on 10th April in 2001 and 24th April 2003, the samples were taken on 23rd May, 15th June, 14th July in 2001, and 4th June, 18th June and 23rd July in 2003.

Analytical methods. The substrate-induced respiration (SIR), dissolved organic carbon and nitrogen (DOC and DON), and soil pH were determined from the soil samples. DOC concentration was used regularly to quantify DOM content of soils.

The SIR determination was carried out according to Anderson and Domsch (1978). 20 g of soils were displaced to about 150 cm^3 volume bottles. Formerly, the soils were set to 50% of the water capacity and incubated at 22°C for 7 days. At the day of analysis, 0.5 cm^3 100 mg/cm^3 glucose solution, which corresponds to 40 mg C/cm^3, was added to soils and mixed thoroughly. Samples were incubated then at 22°C, and the CO_2 concentration was measured after 30 and 150 min.

The CO_2 concentration was determined as methane by gas chromatography with FID detector. The SIR values were corrected with CO_2 and HCO_3 in the gas phase and in the soil solution (Sparling and West 1990). The SIR was calculated from difference between the CO_2 concentration of 30 and 150 min. The MBC was estimated from SIR with factor 30 (Kaiser et al. 1992).

Table 3. Treatments applied

Treatment	Lime dose (g/kg)	Lime dose (t/ha)
Control	0 g	0
L_1	1 g calcite (= half the calculated rate)	1.4
L_2	2 g calcite (= calculated rate)	2.8
L_3	3 g calcite (= one and a half times the calculated rate)	4.1

*rates of ameliorant were calculated from y_1 and K_A (Győri and Rédly 1988)

Table 4. The effect of liming on soil microbial biomass-C (mg/kg) and soil pH (in brackets) on Kisvárda soil and on Nyírlugos soil (pot experiment, taking six samples from each soil over two vegetation periods)

	Kisvárda soil					
	18 May 2000	14 June 2000	21 July 2000	23 May 2001	15 June 2001	14 July 2001
Control	29^{a} (4.5)	41^{a} (4.3)	51^{a} (4.4)	26^{a} (4.0)	46^{a} (3.9)	42^{a} (3.9)
L_1	70^{cd} (5.4)	82^{b} (5.4)	89^{d} (5.4)	50^{b} (5.0)	70^{bc} (5.3)	60^{b} (4.9)
L_2	106^{e} (6.1)	111^{c} (6.1)	78^{c} (6.2)	62^{c} (6.0)	72^{bc} (6.1)	64^{b} (5.8)
L_3	80^{d} (6.5)	76^{b} (6.5)	67^{b} (6.5)	62^{c} (6.4)	81^{c} (6.4)	55^{b} (6.2)
	Nyírlugos soil					
	23 May 2002	20 June 2002	18 July 2002	4 June 2003	18 June 2003	23 July 2003
Control	34^{a} (4.4)	38^{a} (4.1)	37^{a} (4.1)	41^{a} (4.1)	43^{a} (4.3)	32^{a} (4.3)
L_1	64^{bc} (5.2)	57^{b} (5.7)	56^{b} (5.0)	59^{b} (4.7)	65^{b} (4.9)	56^{bc} (4.8)
L_2	110^{d} (5.7)	88^{c} (5.8)	59^{b} (5.9)	64^{b} (5.7)	81^{c} (5.7)	71^{d} (5.8)
L_3	75^{c} (6.4)	64^{b} (6.3)	59^{b} (6.3)	56^{b} (6.4)	72^{bc} (6.3)	50^{b} (6.3)

$^{a-e}$indicates significant differences within each column at the 5% level of probability according to Duncan test. For treatments: see Table 3

Prior to the DOC and DON analysis, 15 g soil samples were extracted in 0.01M $CaCl_2$ for 2 h with 1:10 soil:solution ratio (Jászberényi et al. 1994), and then filtered through a 0.45µM membranes. The DOC and DON concentrations were determined from this solution. The DOC content was determined by TOC analyser, DON by measuring the difference between total dissolved nitrogen and inorganic nitrogen ($NO_3^- + NH_4^+$) as described by Houba et al. (1994).

The soil pH was measured in aqueous suspension (1:2.5 ratio of soil to water) following 24 h sedimentation at room temperature by glass electrode. Hydrolytic acidity (y1) was measured by titration the soil samples with Ca-acetate at pH 8.2. The soil texture analysis was done by pipette method, the CEC of soils was determined after a non-buffered 0.1M $BaCl_2$ saturation. Organic carbon was measured by oxidation with $K_2Cr_2O_7$ according to the method of Tyurin, whilst N content was determined after $H_2SO_4 + H_2O_2$ digestion according to the modified method of Kjeldahl.

RESULTS AND DISCUSSION

Changes in MBC as affected by liming. Liming was found to increase soil respiration and consequently MBC, but at the maximum lime rate these values stagnated or declined in many cases on both soils (Table 4).

Calculating the increment between control and the maximum in percentage due to liming, the increase were 365, 270 and 174% on the Kisvárda soil at the first growing season, respectively, and these values belong to L_2, L_2 and L_1 calcite levels. For Nyírlugos soil values are: 323, 231 and 159%, respectively. Maximums were measured in the L_2, L_2 and L_2–L_3 treatments.

In the second year of the experiments, the effects of treatments were much lower both on Kisvárda and on Nyírlugos soils. The percentage increments were 238, 176 and 152% in Kisvárda, as well as 156, 188, 222% in Nyírlugos. With an exception of one sampling (KV, 5th sampling), maximum values were reached at L_2 treatment.

On each soil, pH of soils was altered significantly and systematicly by liming through the whole growing periods. Pattern of this change is different from that obtained for MBC. Maximum pH values were measured at maximum lime rate, and it increased the control pH with 2 units.

The positive liming effect on the amount of microbial biomass may be considered with respect to three factors, namely:

(i) There may be a significant increase in pH after liming application involving a decline in toxic element concentration (Al, Mn) as Al and Mn precipitate as hydroxides (Castro Filho and Logan 1991).

(ii) More favourable pH conditions of soil cause higher plant and root biomass production and as a consequence of that an increase appeared in concentrations of organic compounds released by roots into the soil solution (Smith 1976). These dissolved organic compounds are primary substrates for microorganism (Jones et al. 2005). Growing amount of substrate creates circumstances in which microorganisms can increase their number.

(iii) DOM content of soil may increase in other way: increasing pH induced by the application of

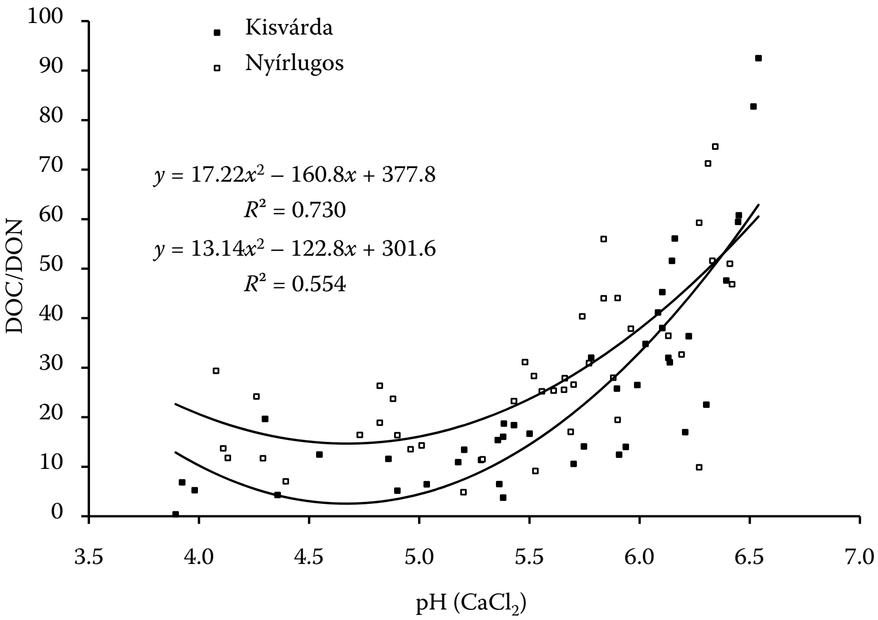

Figure 1. Correlation between soil pH and the ratio of dissolved organic C to N (DOC/DON)

lime materials causes the net negative charge of the molecule increased, thus leading to a steric conformational change of that. At high pH, DOM molecules are in an expanded structure, because charges endeavour to situate themselves as far apart as possible. Owing to the expanded structure and the net negative charge, the molecule can be fully penetrated by water (Tombácz and Rice 1999), therefore it becomes hydrophilic. As a result, the bonding between organic compounds and soil particles decreases, making organic substances more available for microbial consumption (Curtin et al. 1998).

However, there is still a question why the MBC content decreases at the end of the vegetation period. This can be attributed to the fact that at high lime rates the N supplies to microorganisms are insufficient due to the small quantity of rapidly mineralisable organic matter in the soil. Although with increasig pH, DOM concentration increased, N content of that decreased drastically in the range of pH 5.5–6.5, due to the microbiological decay (Figure 1). This pH range corresponds to that which was obtained in L_2 and L_3 treatments. Because of this the ratio of DOC/DON began to increase exponentially at L_2 and L_3 treatments.

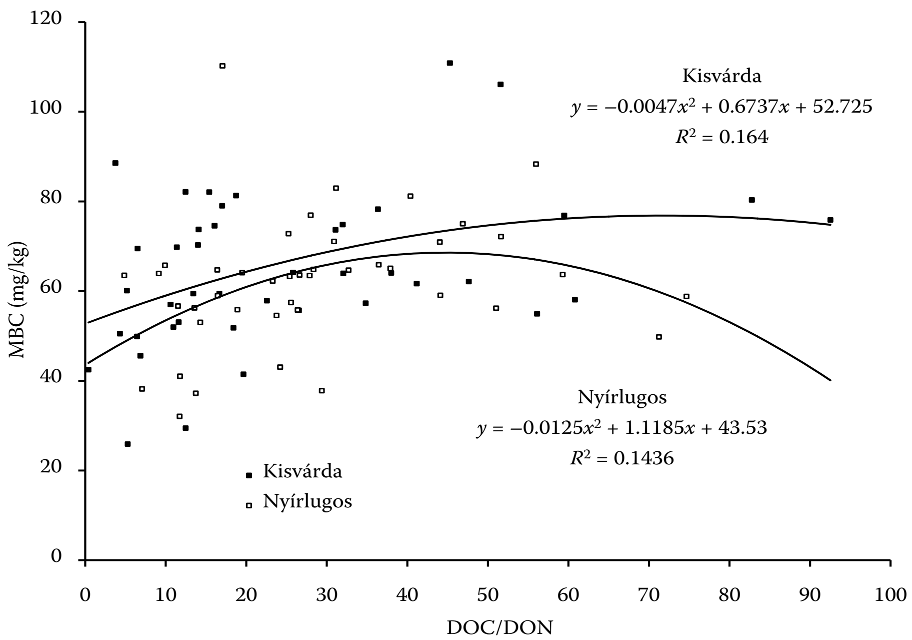

Figure 2. Correlation between the ratio of water-extractable organic C to N (WEOC/WEON) and the microbial biomass C content in the soil

Table 5. Average microbial biomass carbon (MBC) content of soils during the growing seasons

	Kisvárda					
	18 May 2000	14 June 2000	21 July 2000	23 May 2001	15 June 2001	14 July 2001
MBC	71^{b}	77^{c}	71^{b}	50^{a}	67^{b}	55^{a}
	Nyírlugos					
	23 May 2002	20 June 2002	18 July 2002	4 June 2003	18 June 2003	23 July 2003
MBC	70^{b}	61^{b}	52^{a}	55^{a}	65^{b}	52^{a}

$^{a–c}$indicates significant differences within each column at the 5 % level of probability according to Duncan test

The DOC/DON ratio increased by adding lime materials from the initial values 10–20 to 80–90, which indicates a significant decrease in the N content of DOM. It could be deduced from this that the DOC/DON ratios are good indicators for changing N supply ability of the soil.

In tune with this, there was a relationship between the ratio of dissolved organic C to N and the microbial biomass-C (Figure 2). At early stage, the increase in DOC/DON ratio caused an appreciable increase in MBC, but after this increase, MBC stagnated and declined.

Under the given experimental conditions the DOC/DON ratio rose to above 30–40, causing disturbances in N supplies to microorganisms. The N content of the readily mineralisable organic matter in the soil became so low that it inhibited the reproduction of the microorganisms. Persson et al. (1989) suggested that liming increased net N mineralization in forest soils with low C to N ratios (< 30) and reduced net mineralization in soils with large C to N ratios (> 30). It follows that the dissolved organic matter (DOM) could be an important indicator for environmental changes, and could be used to characterize the short-term N-supplying ability of the soil.

Changes in MBC content with time. A marked trend was found in changes of soil MBC content over two vegetation periods in the average of treatments (Table 5).

Maximum values of MBC were measured in the middle of growing season (2nd and 5th sampling) on both soils. Maximum values were significantly distinct from the values measured at the beginning and at the end of the growing period – excluding the 1st and 2nd sample on the Nyírlugos soil.

Necessary condition for microorganisms to grow their number up is sufficient substrate. The dissolved organic matter (DOM) is considered as primary substrate for microorganism, moreover plant roots are known to excrete highly labile compounds, such as carbohydrates and amino acids (Eviner and Chapin 1997) increasing the concentration of DOM molecules in the soil solution.

The amount of organic compounds released from roots is particularly great in the growing season (Paul and Clark 1996) and maximum yield of root attained in the middle of the growing period in our experiment, too (data not shown). Relationship was found between soil respiration, MBC and root biomass by Lu and co-workers (2002). Because of these facts, it can be concluded that the highest MBC values found in the middle of the growing period can be explained by increased root matter, thereby leading to a higher DOM concentration.

Because of a decrease of the root biomass, less DOM substances released in turn of June to July causing a trouble in C- and N-supply to microorganisms. However, dynamics of DOC did not show

Table 6. Dissolved organic carbon (DOC and DON; mg/kg) and ratio of DOC to DON

	Kisvárda					
	18 May 2000	14 June 2000	21 July 2000	23 May 2001	15 June 2001	14 July 2001
DOC	276^{d}	184^{c}	144^{bc}	134^{b}	129^{b}	99^{a}
DOC/DON	41^{c}	43^{c}	26^{ab}	21^{a}	29^{b}	23^{a}
	Nyírlugos					
	23 May 2002	20 June 2002	18 July 2002	4 June 2003	18 June 2003	23 July 2003
DOC	131^{a}	197^{b}	139^{a}	176^{b}	218^{c}	198^{b}
DOC/DON	19^{a}	43^{d}	37^{c}	26^{b}	33^{b}	35^{ab}

$^{a–d}$indicates significant differences within each line at the 5 % level of probability according to Duncan test. DOC – dissolved organic carbon; DON – dissolved organic nitrogen the same trend as MBC for the Kisvárda soil, it was a continuous decline over the two vegetation period. For the Nyírlugos soil, maximum values of DOC were measured in the middle of growing season (2nd sampling in June) corresponding with root biomass dynamics.

Seasonal dynamics of MBC can be truly interpreted by the changes in C to N ratio of dissolved organic matter (Table 6).

The DOC/DON ratio reached maximum in the middle of the growing period indicating that microbial decay of N was also at maximum. Because of mineralization the DOM was getting poor in N and it further interfered gaining upon microbes.

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