Relationships between quality and quantity of soil labile fraction of the soil carbon in Cambisols after liming during a 5-year period

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ABSTRACT

The labile fraction of soil organic carbon (SOC) in terms of its quantity is a sensitive but dynamic indicator of the reactive agent in soils. If it is to be considered as a feature of soil quality, the value of its quantity should be completed by data on its quality. It can be expressed by the value of the rate constant of microbial oxidation k_{bio} of this fraction or by data on chemical stability during hydrolysis or oxidation. If the quality of SOC labile fraction is not determined, at least the ratio of C_{MIC}: C_{org} should be given. The adjustment of soil acidity increases the microbial activity of soils, therefore the quantity of SOC labile fraction decreases and at the same time k_{bio} decreases proportionately to the increasing stability of soil organic matters. During a 5-year period after the liming the soils acidify again and this process passes the faster the lesser their ion exchange capacity and buffering are. The quantity of the labile fraction of SOC raises again, its stability decreases and k_{bio} raises again. The conversion pH value has secondary relevance.

Keywords: soil organic carbon (SOC); labile fraction SOC; quality and quantity; effect of soil reaction adjustment; time changes

The easily decomposable fraction of soil organic matter (SOM) is a reactive agent with a relatively high transformation rate (Ghani et al. 2003). Its content is proportional to the quantity of microbial biomass in soil (Lovell and Jarvis 1998) and therefore it is an important factor of potential soil fertility. Many authors consider the higher content of the labile fraction of the soil carbon (SOC) as a feature of soil quality (Ghani et al. 2003, Haynes 2005, Maia et al. 2007). Unfortunately, it cannot be stated that it is a steady feature of quality. Labile SOC transformation depends on the microbial activity of soil microorganisms, and so the rate of a quantitative change in the determined quantity of labile fraction is very different and related to pH (Brady and Weil 1999), soil aeration, moisture, temperature, availability, and composition of C and N substrates (Marschner and Kalbitz 2003). Hence, the determination of the quantity of SOC labile fraction as a feature of soil quality is somewhat unreliable and parallel determination of the carbon of soil microbial biomass and its metabolic efficiency may be useful (Uhlířová et al. 2005). The ratio of microbial biomass carbon to total organic carbon C_{MIC}:C_{org} is considered as an important indicator of soil microbial activity. The labile fraction of SOM is described in a very different way (Kolář et al. 2009).

The lability of soil organic matter is deduced from the content of carbon C_{PM} oxidizable with a neutral solution of 333 or 33 mmol $KMnO_4$ (Blair et al. 1995).

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Reviewing the number of methods for determination of SOM labile fraction, the determination of 'active carbon' (Körschens et al. 1990) as hot water extractable carbon C_{hws} is used the most frequently. This generous choice of the methods is related only to the quantity of the labile fraction of soil carbon.

It would be very convenient if, besides the quantity of SOC labile fraction, its quality were also measured. It is already predetermined by the choice of the method to some extent, e.g. the degree of lability increases in the sequence $C_{PM} < C_{hws} < C_{cws}$, but quality can also be deduced from the values of basal respiration which besides the availability of organic matters in the soil solution shows the capacity of present microorganisms to utilize them and it may vary in different environments. The first contribution to the measurement of quality of the labile fraction of soil organic carbon besides the measurement of quantity was the attempt to divide this fraction according to the results of sequential oxidation or hydrolysis. For the classification of soil sample C_{ox} according to the degrees of lability the original Walkley-Black method (1934) is used in modification with solutions in which the H_2SO_4 concentration varies at the stable concentration of $K_2Cr_2O_7$ (Chan et al. 2001). Hydrolysis with strong mineral acids of different concentration is also used for lability determination (Shirato and Yokozawa 2006). Tirol-Padre and Ladha (2004) attempted to determine the real quality of labile SOC by measuring the kinetics of SOC oxidation with 33 mmol neutral $KMnO_4$ at room temperature. We found out that the determination of the rate constant k_{chem} of this oxidation showed excellent reproducibility but it corresponded to the proportion of C_{PM} in total C_{ox} rather than to the degree of SOC lability (Kolář et al. 2009). This is the reason why we prefer to express the quality of SOC labile fraction by the rate constant of biochemical oxidation k_{bio} of C_{hws} fraction derived from the kinetic study of several determinations of biochemical oxygen demand during 20 days (Kolář et al. 2006, 2009). k_{bio} is more sensitive to the degree of SOC lability but because it is determined from biochemical oxidation its reproducibility is worse than in k_{chem}. In the presented paper we tried to express the importance in determining the quality of labile fraction of SOC, excluding its quantity using the example, of the changes of this fraction in Cambisols under permanent grassland in a foothills region after soil reaction adjustment by calcification.

MATERIALS AND METHODS

Study site. Differences were studied in the quantity of SOM labile fraction expressed as C_{hws} (hot water soluble carbon) and in its quality expressed by the value of the rate constant of biochemical oxidation of this fraction in relation to time, altitude above sea level, fertilization and adjustment of the acid soil reaction by liming with a mixture of $Ca(OH)_2 + CaCO_3$ applied to a permanent grassland on sandy-loam Cambisol (CM) of grassland types Arrhenatherion I – in localities Besednice (610 m a.s.l), Malonty (690 m a.s.l), Brloh (730 m a.s.l.) and Světlík (790 m a.s.l.). In these localities sampling sites were stabilized exactly where soil samples were taken in 2003–2008 from the *Galium pumilum*, *Arrhenatherum elatius*, *Hypericum maculatum*, *Achillea millefolium*, *Agrostis tenuis*, *Vicia cracca*) and Arrhenatherion II (*Plantago lanceolata*, *Campanula patula*, *Galium pumilum*, *Agrostis tenuis*, *Holcus mollis*, *Alchemilla vulgaris*, *Hypericum maculatum*) in a foothills region of South Bohemia profile of 0–0.15 m in monthly intervals in the months of April to November. Temperatures and precipitation amounts were registered at the same time.

These fertilization treatments were used every year: 0, NPK, NPK + liming with a mixture of $CaCO_3 + Ca(OH)_2$ whereas liming in the liming treatment only and in the NPK + liming treatment was done only once at the beginning of the whole experiment. The influence of liming was investigated since 1 year after liming. Doses of NPK/1 000 m^2: 10 kg N + 10 kg K + 10 kg P in the form of NPK fertilizer. The dose of Ca-compounds [$Ca(OH)_2 + CaCO_3$ at a 1:1 ratio]: 300 kg/1000 m^2.

Chemical analysis. The value of pH_{KC1}, content of available nutrients (Mehlich 1984), ion-exchange capacity of soil T, sum of exchangeable cations and degree of sorption saturation V (Staňa et al. 1992), total organic carbon C_{org} (ISO 10694) and oxidizable carbon C_{ox} (ISO 14235) were determined to describe the soils of experimental localities.

The labile fraction of soil organic carbon SOC was described by determination of labile carbon C_{PM} (Blair et al. 1995) and hot water extractable carbon C_{hws} (Körschens et al. 1990). The quality of C_{hws} fraction was determined by the rate constant of its biochemical oxidation k_{bio} (Kolář et al. 2009).

Stable organic matters were determined as carbon of humic acids C_{HA} and carbon of fulvic acids C_{FA} and based on these values, the apparent degree of humification $S_H = C_{ox\ HA} + C_{ox\ FA}/C_{ox\ tot}$ was found out (humins are not represented). Total nitrogen N_{tot} and mineral nitrogen N_{min} were also determined. Microbiological analyses comprised the determination of microbial biomass carbon C_{MIC} (Vance et al. 1987) and from this value the microbial quotient $MQ = C_{MIC}/C_{org}$ was calculated.

Statistical analysis. Data were analyzed using Statistica 5.5 for Windows (Statsoft, USA). The effects of altitude above sea level, temperatures, precipitation amount, grassland type, NPK application, sample collection date were tested by the analysis of variance (two-way ANOVA). Lord's test, based on the R range of parallel determinations (Sachs 1974), was used to calculate the reliability interval of the mean at a significance level $\alpha = 0.05$.

RESULTS AND DISCUSSION

Table 1 shows the characteristics of the sites where experiments were conducted. The soils are sandy-loam, acid, with a low content of available nutrients, low ion-exchange capacity and low degree of sorption saturation, low microbial activity, higher content of SOC and its labile forms.

The mathematical and statistical evaluation of results proved that the dynamics of the formation and disappearance of labile organic matters composing the C_{hws} fraction is so high that differences in the instantaneous quantity of C_{hws} in sandy-loam Cambisol with permanent grassland are statistically insignificant (the criterion $P < 0.05$) among the treatments

Table 1. The analysis of soils (sandy-loam Cambisols) in the profile of 0–0.15 m in experimental localities. The means of eight annual samplings in 2003–2008 are shown

Locality		Besednice	Malonty	Brloh	Světlík
Altitude a.s.l. (m)		610	690	730	790
Content of particles < 0.01 mm (%)		23	27	21	25
pH_{KCl}		5.64	5.47	5.05	5.23
Available nutrients (Mehlich 1984) (mg/kg)	P	22	28	20	16
	K	49	60	38	45
	Ca	390	410	175	240
	Mg	18	20	15	11
Ion-exchange cation capacity T (Gillman 1979) (mmol chem. eq./kg)		121	83	58	55
Exchange cations S (Gillman 1979) (mmol chem. eq./kg)		83.5	52	24	27.5
Degree of sorption saturation V (%)		69	63	42	50
Degree of humification $S = \frac{C_{ox\,HA} + C_{ox\,FA}}{C_{ox\,tot}} \times 100$ (%)		28.7	18.9	8.3	10.7
Total nitrogen N_{tot} (g/kg)		1.95	2.03	2.32	2.85
Mineral nitrogen N_{min} (g/kg)		0.02	0.02	0.01	0.02
Total organic carbon C_{org} (ISO 10694) (g/kg)		28.2 ± 4.8	24.5 ± 5.1	32.3 ± 3.5	26.0 ± 3.0
Oxidizable carbon C_{ox} (ISO 14235) (g/kg)		25.9 ± 4.6	22.7 ± 4.9	31.0 ± 3.7	24.2 ± 3.5
Labile carbon C_{PM} (Blair et al. 1995) (g/kg/h)		5.25 ± 1.05	5.80 ± 1.25	7.75 ± 1.63	5.63 ± 0.88
Labile carbon C_{hws} (Körschens et al. 1990) (g/kg)		0.97 ± 0.32	1.33 ± 0.45	1.99 ± 0.60	1.53 ± 0.55
Rate constant of biochemical oxidation k_{bio} (Kolář et al. 2009) (24 h)		0.024 ± 0.005	0.025 ± 0.007	0.083 ± 0.010	0.080 ± 0.008
Carbon of microbial biomass C_{MIC} (Vance et al. 1987) (g/kg)		0.652 ± 0.305	0.700 ± 0.419	0.445 ± 0.288	0.510 ± 0.321
Microbial quotient $MQ = \frac{C_{MIC}}{C_{org}}$		2.31×10^{-2}	2.85×10^{-2}	1.38×10^{-2}	1.96×10^{-2}
$\frac{C_{hws}}{C_{org}} \times 100$ (%)		3.43	5.42	6.16	5.88

Table 2. Average values of changes in the rate constant of biochemical oxidation of C_{hws} fraction and changes in C_{hws}, C_{MIC} and the degree of humification S after adjustment of the acid soil reaction by liming (liming 9/2003, analysis of soil samples 9/2004)

Locality	Besednice	Malonty	Brloh	Světlík
pH KCl	5.98	6.10	6.15	6.24
Labile carbon C_{hws} (Körschens et al. 1990) (g/kg)	0.35 ± 0.07	0.78 ± 0.12	1.51 ± 0.45	1.25 ± 0.28
Difference in C_{hws} content caused by soil reaction adjustment (g/kg)	−0.62	−0.55	−0.48	−0.28
Carbon of microbial biomass C_{MIC} (Vance et al. 1987) (g/kg)	0.835 ± 0.324	1.045 ± 0.495	0.794 ± 0.371	0.992 ± 0.528
Difference in C_{MIC} content caused by soil reaction adjustment (g/kg)	+0.183	+0.345	+0.349	+0.482
Rate constant of biochemical oxidation k_{bio} (Kolář et al. 2009) (24 h)	0.020 ± 0.003	0.017 ± 0.002	0.051 ± 0.007	0.043 ± 0.007
Difference in k_{bio} value caused by soil reaction adjustment (24 h)	−0.004	−0.008	−0.032	−0.037
Degree of humification S (%)	28.6	18.9	8.2	10.9
Difference in S value caused by soil reaction adjustment (%)	−0.1	0.0	−0.1	+ 0.2

with different altitude above sea level (610–790 m a.s.l.) also with different temperatures and precipitation amounts in the particular altitudinal localities. They are also statistically insignificant with regard to grassland type (Arrhenatherion I, II) and date of sample collection in the months of April–November. Neither did the NPK application bring an expected change in statistically significant differences, obviously because the humid environment of experimental localities, low soil pH and high permeability of lighter soils at the sites of experiments were the causes of very low nitrogen utilization as was demonstrated by the observed elutions of mineral nitrogen in buried lysimeters. Only decreases in the quantity of C_{hws} fraction were statistically significant after adjustment of the acid soil reaction by liming as a consequence of improvement in conditions for the activity of soil microorganisms as could be expected.

A change in the quality of C_{hws} fraction, expressed by the rate constant of its biochemical oxidation, is an important result of this part of the paper. After the soil reaction adjustment and reduction in the quantity of matters composing the C_{hws} fraction there is a simultaneous decrease in the values of the rate constant of its biochemical oxidation, which implies a decrease in labile components and an increase in

Table 3. Average changes in C_{MIC} content, microbial quotient MQ and labile carbon fraction C_{hws} 5 years after the soil reaction adjustment (liming 9/2003, analysis of soil samples 9/2008)

Locality	Besednice	Malonty	Brloh	Světlík
Carbon of microbial biomass C_{MIC} (Vance et al. 1987) (g/kg)	0.810 ± 0.389	0.865 ± 0.405	0.510 ± 0.233	0.482 ± 0.254
Max. difference in C_{MIC} after soil reaction adjustment and after 5 years (g/kg)	−0.025	−0.180	−0.284	−0.510
MQ	2.05×10^{-2}	1.94×10^{-2}	1.30×10^{-2}	1.31×10^{-2}
Difference in MQ	$2.30 - 2.05 = = -0.25 \times 10^{-2}$	$2.60 - 1.94 = = -0.66 \times 10^{-2}$	$2.08 - 1.30 = = -0.78 \times 10^{-2}$	$2.83 - 1.31 = = -1.52 \times 10^{-2}$
Labile carbon C_{hws} (Körschens et al. 1990) (g/kg)	0.45 ± 0.11	0.88 ± 0.25	1.83 ± 0.58	1.45 ± 0.49
Max. difference in C_{hws} after soil reaction adjustment and after 5 years (g/kg)	+0.10	+0.10	+0.28	+0.20

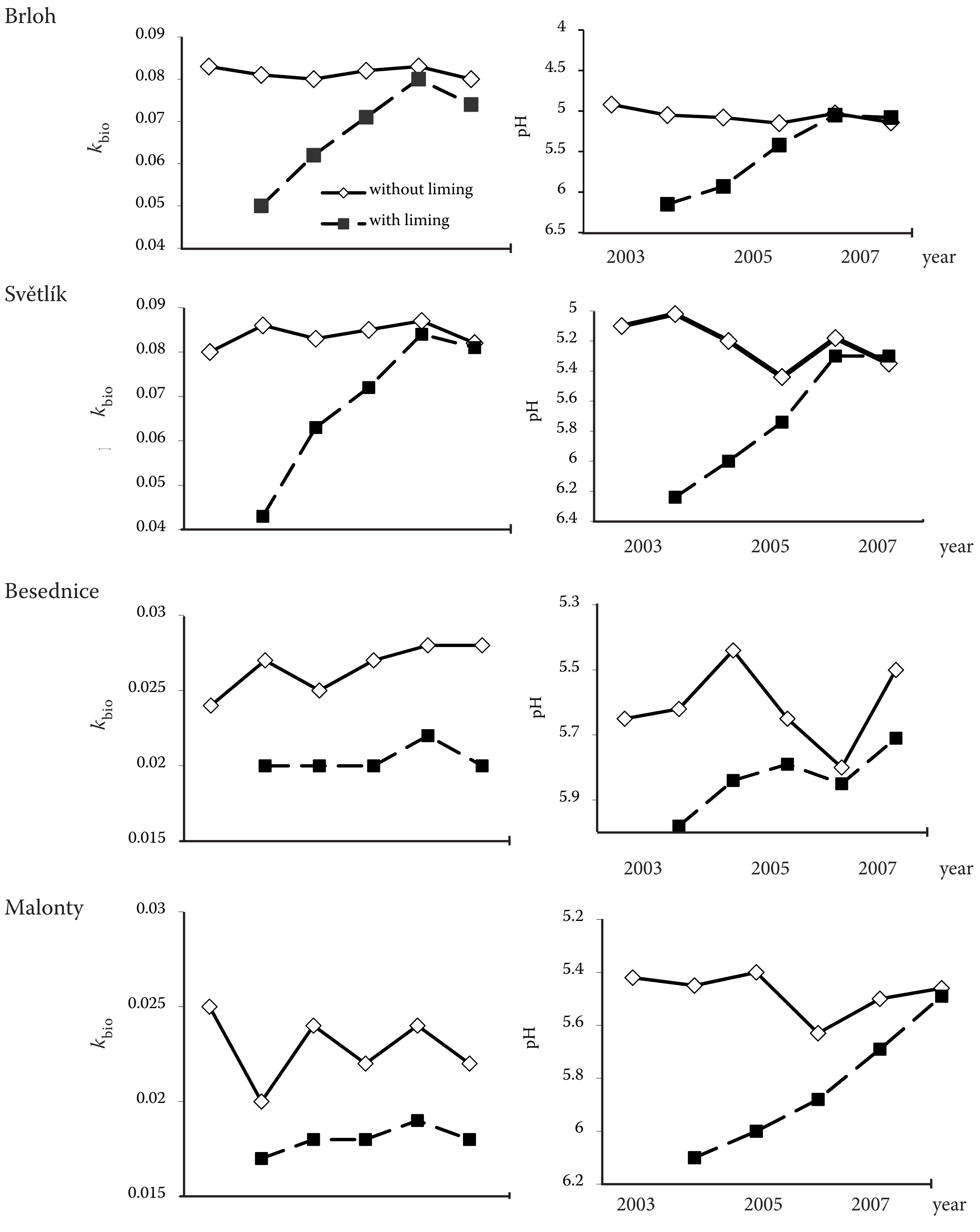

Figure 1. The average value of k_{bio} (Kolář et al. 2009) during the experiment in the particular localities in treatments with and without liming (pH adjustment was done in September 2003)

stable components of this fraction, i.e. a change in their quality (Table 2). A change in the locality Brloh and Světlík is especially apparent (Figure 1).

Although an increase in the values of C_{MIC} and microbial quotient MQ implies the higher activity of studied soils after adjustment of the soil reaction that is reflected in higher consumption of the C_{hws} labile fraction and increased stability of its residue as revealed by a decrease in k_{bio}, the degree of humification has not changed at all. Obviously, changes take place only in the category of the degree of primary soil organic matter lability.

Table 4. Distribution of organic matter of soils in experimental localities of the fraction 2.00–0.25 mm and fraction < 0.25 mm in % of total C_{org} in dry matter into classes by hydrolyzability according to Chan et al. (2001) after the soil reaction adjustment and of the fraction 0.25 mm 5 years after the soil reaction adjustment

	Fraction (%)											
Particle size (mm)	2.00–0.25				< 0.25				< 0.25 (after 5 years)			
Locality	1	2	3	4	1	2	3	4	1	2	3	4
Besednice	31	23	28	18	25	34	16	25	35	25	19	21
Malonty	35	21	26	18	29	31	16	24	33	27	19	21
Brloh	35	24	25	16	38	30	14	18	40	30	13	17
Světlík	34	25	24	17	36	28	16	20	41	28	16	15
$lsd_{0.05}$	6.7	3.4	3.5	2.2	9.9	5.4	2.6	2.1	10.1	4.7	2.4	2.2

Fraction 1 = 6 mol/L H_2SO_4; Fraction 2 = 9–6 mol/L H_2SO_4; Fraction 3 = 12–9 mol/L H_2SO_4; Fraction 4 = C_{org} – 12 mol/L H_2SO_4

However, in the course of time the change in the quality and quantity of C labile fraction gradually balances up, probably with gradual reacidification of the site, reinhibition of the soil microedaphon activity and mainly with the production of new, yet unconsumed more labile matters of the C_{hws} fraction (Figure 1, Table 3). An addiction of k_{bio} and pH is totally evident in soils in localities Brloh and Světlík, it means in soils that differ more in value ion exchange capacity and less in pH value.

Table 3 documents the average total change in the studied values for the whole experimental period under the influence of the soil reaction adjustment.

Figure 1 illustrates that the rate constant of biochemical oxidation of C_{hws} fraction, as a feature of the quality of this fraction, does not increase linearly after the soil reaction adjustment, hence the lability of the C_{hws} fraction increases at the end of the experimental period, and such an increase occurs later if the ion-exchange capacity of soil in the given locality is higher.

Table 3 shows that ecological conditions of the site predetermine the permanent average level of studied values and the resumption of the original level is faster if the ion-exchange capacity, and so the buffering capacity of soil, is lower. The influence of the original pH_{KCl} level before liming seems to be less significant.

We verified this change in the degree of the C_{hws} fraction lability purely chemically by an oxidation method according to Chan et al. (2001) and the results fully confirmed our assumptions (Table 4).

The proneness of organic matter to oxidation is mostly identical with the organic matter lability in aerobic and anaerobic microbial processes even though it is not a rule. In our experiment the chemical oxidizability of soil organic matter is fully identical with its lability expressed by an increase in the rate constant k_{bio} of its biochemical oxidation only in the particle-size fraction < 0.25 mm. Differences are statistically insignificant in the particle-size fraction 2.00–0.25 mm and this is the reason why the measurement in this particle-size fraction 5 years after the soil reaction adjustment is not shown in Table 4.

Five years after calcification was carried out the soil in Brloh locality showed the highest value of k_{bio}, MQ was the lowest and the proportion of C_{hws} in total C_{org} was the highest there (Table 1), and it also had the highest amount of the most labile

Table 5. Distribution of organic matter of soils in experimental localities in % of total carbon in dry matter into classes by hydrolyzability according to Rovira et Vallejo (2000, 2002) after soil acidity adjustment and 5 years after this adjustment

Locality	Fraction (%)		
	labile	partly resistant	resistant
Besednice	20 ± 2	21 ± 3	59 ± 16
After 5 years	22 ± 4	20 ± 3	58 ± 14
Malonty	16 ± 5	16 ± 4	68 ± 13
After 5 years	20 ± 4	16 ± 4	64 ± 15
Brloh	15 ± 5	35 ± 8	50 ± 11
After 5 years	26 ± 4	36 ± 9	38 ± 10
Světlík	14 ± 5	34 ± 9	52 ± 12
After 5 years	24 ± 6	33 ± 8	43 ± 10

fraction 1 in chemical oxidation (Table 4). Except the value of MQ the situation in Besednice locality was opposite. Five years after the adjustment of soil reaction the resumption of the original content of labile fractions of soil carbon is evident in the fraction < 0.25 mm but the relation to the k_{bio} values was maintained again (Table 4, Figure 1).

Because the C_{hws} fraction accounts only for a minute part of C_{ox} of soil samples, the dynamics of changes, formation and disappearance of labile and stable fractions of soil organic matter may also comprise more stable, enzymatically hydrolyzable fractions of soil organic matter that according to the methodology of C_{hws} isolation are beyond the reach of determination of this fraction. Therefore for all soil organic matter of our treatments we used the determination of labile, semi-stable and resistant fractions by a hydrolytic method of Rovira and Vallejo (2000, 2002) in Shirato and Yokozawa's (2006) modification. The results confirmed our preceding conclusions less distinctly but statistically significantly (Table 5).

This table demonstrates the resumption of the original state of soil carbon fractions before the soil reaction adjustment; five years after this measure was taken the content of labile fraction was increasing again, the content of stable fraction is decreasing while the largest differences are in soils with the lowest value of ion-exchange cation capacity T and regardless of the pH_{KCl} value of soils that was reached by soil acidity neutralization.

These conclusions may be drawn:

The labile fraction of C_{hws} in terms of its quantity is a sensitive indicator of the reactive agent in soils but it is always a dynamic value depending on the ratio of the formation to disappearance rate of this fraction.

If the quantity of C_{hws} is to be considered as a feature of potential soil fertility as claimed by many authors, either its quality should always be defined on the basis of determination of the rate constant of its biochemical oxidation from reaction kinetics of microbial oxidation or at least its stability should be determined from the results of its gradual chemical oxidation according to Chan et al. (2001).

If the quality of C_{hws} fraction is not determined besides its quantity, it is necessary to study at least the features of microbial transformation of the labile carbon fraction by determination of the carbon of soil microbial biomass and its metabolic efficiency and the ratio of microbial biomass carbon to total organic carbon C_{MIC} : C_{org}, as recommended by Uhlířová et al. (2005).

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