

Relationship Between Soil Organic Carbon and Microbial Biomass on Chronosequences of Reclamation Sites

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Abstract. The interrelationship between soil microorganisms and soil organic carbon was studied on an agricultural and on a forest chronosequence of open-pit mine reclamation soils. Thirty years after reclamation, soil carbon levels of 0.8% on the agricultural sites and 1.7% on the forest sites (A-horizon) were reached. Microbial biomass rose very fast to levels characteristic of undisturbed soils. Microbial carbon (C_{micr}) was $57 \text{ mg} \cdot 100 \text{ g}^{-1}$ soil after 15 years on the agricultural sites and $43 \text{ mg} \cdot 100 \text{ g}^{-1}$ on the forest sites. The contribution of C_{micr} to the total organic carbon (C_{org}) decreased with time, more rapidly on the forest sites than on the agricultural ones. From the $C_{\text{micr}}/C_{\text{org}}$ ratio it became evident that both chronosequences had not yet reached a steady state within the 50 years of reclamation. A significant decrease of the metabolic quotient $q\text{CO}_2$ (microbial respiration per unit biomass) with time was observed on the agricultural sites but not on the forest sites. The $C_{\text{micr}}/C_{\text{org}}$ ratio proved to be a reliable soil microbial parameter for describing changes in man-made ecosystems. For evaluating reclamation efforts, the $C_{\text{micr}}/C_{\text{org}}$ ratio can be considered superior to its single components (C_{micr} or C_{org}) and to other parameters.

Introduction

Land subjected to open-pit mining is usually replaced by overburden materials containing little organic matter [20–23] and demonstrates low levels of microbial activity [24].

Organic matter is an essential component of soil quality [23], and microorganisms play a vital role in the maintenance of microbial fertility [18, 25]. Thus, both organic matter and microbial activity can be regarded as indicators related to the process of soil genesis on minesoils [20]. Enzyme activities [14, 24], soil respiration [16, 26], and ATP content [10] have been found to be lower in minesoils than in native soil. The same has been found for biomass and diversity of microorganisms [26].

Little attention has been given to the time course of recovery. Stroo and Jencks [24] measured enzyme activities and soil respiration in 11 minesoils

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(aged 3–20 years). Schafer et al. [20] studied soil genesis, water relations, and microbial activities (ATP levels) of 1- to 50-year-old minesoils. They also studied the dynamics of organic matter accumulation in minesoils, as did Skujins and Richardson [23].

There are indications that the recovery of different parameters follows different kinetics in reclaimed minesoils, e.g., microbial properties may reach the level of native soils faster than total organic matter content [20]. Parameters on a higher integration level could, therefore, be more useful in describing minesoil development than single parameters.

We followed two approaches. The first is based on the ratio of the soil microbial biomass to the total organic carbon of a soil. The basic carbon and energy source for heterotrophic production is the carbon input from net primary production (NPP). As long as NPP exceeds respiration of heterotrophs (R) in any given ecosystem, organic matter will accumulate; as soon as R equals NPP, a steady state will be reached [9]. When steady-state conditions are achieved, the proportion of microbial biomass carbon (C_{micr}) in the total soil organic carbon (C_{org}) will, at least in agricultural ecosystems, equilibrate at a characteristic level. Deviations from this level would indicate that the soil is either losing or accumulating carbon [3, 5, 11]. Thus, if the equilibrium constant is known, the actual $C_{\text{micr}}/C_{\text{org}}$ ratio of a disturbed or reclaimed soil should provide valuable information on how near the soil is to its final state.

The second approach is derived from Odum's theory of ecosystem succession [17]. Odum proposed that the ratio [total respiration (R)]/[total biomass (B)] decreases with time or succession in an ecosystem. Since the main part of the energy derived from NPP passes through the saprovores system into the dead organic matter pool, where it becomes available for microbial consumption, it is possible to simplify Odum's model by replacing total respiration and total biomass with basal microbial respiration and microbial biomass. Following Anderson and Domsch [2], we express the R/B ratio as the metabolic quotient $q\text{CO}_2$ ($\text{mg CO}_2\text{-C}\cdot\text{hour}^{-1}\cdot\text{mg}^{-1} C_{\text{micr}}$).

The objective of our study was to determine the time required for a forest and an agricultural chronosequence to approach a steady state. We also wanted to examine if the use of certain integrating quotients ($C_{\text{micr}}/C_{\text{org}}$, $q\text{CO}_2$) might be better than the use of single parameters for describing a developing ecosystem or for evaluating reclamation success.

Material and Methods

Sites and Soils

The areas selected for our study are located near Köln, FRG (Rhineland mining district; 51°N, 7°E) and near Helmstedt, FRG (52°N, 11°E). In the Rhine area we chose a chronosequence of reforested soils and in the Helmstedt area a chronosequence of agricultural soils. Detailed data are given in Tables 1 and 2. Both sites belong to lignite strip-mine areas where a large number of minesoils of different age developed on parent material consisting of (or containing large quantities of) loess or loesslike loam. Due to changes in mining practices, the older sites are more heterogeneous than the younger ones, and in some cases the older sites contain large quantities of tertiary sand. During the last decades it has become a common practice to save the top 4 meters of the soils for

reclamation purposes. Since the 1960s, the loess for reforestation in the Rhine area has been mixed with different amounts (20–75%) of gravel to create the so-called Forstkies, a material that is less susceptible to erosion than loess alone.

The climate in both regions is temperate, the average annual rainfall being 641 mm and 628 mm in the Rhine area and near Helmstedt, respectively. The mean annual temperature is 9.6°C and 8.9°C, respectively.

Sampling

On the forest sites the organic layer was removed and samples were taken from the top 15 cm of the mineral horizon; on the agricultural sites the top 15 cm of the A_p horizon were sampled. Three bulked samples, each composed of 10 subsamples, were collected from each site. Some of the sites were sampled twice (see Table 1). After transportation to the laboratory, the soils were sieved (2 mm screen) and the water content was adjusted to a moisture tension of approximately 400 kPa. The samples were stored at 4°C in polyethylene bags with cotton plugs; 5 days prior to analysis the soils were equilibrated at room temperature. Subsamples for the C- and N-determinations were stored at –20°C.

Microbial Biomass Carbon

Microbial biomass (mg · C_{micr} · 100 g⁻¹ soil dry mass) was determined by the method of Anderson and Domsch [1]. The required amount of glucose (C₆H₁₂O₆ · H₂O; mol. wt 198.17) to obtain the maximum initial respiratory response was ground with talcum as carrier and mixed into the soil with an electric mixer (1,600 rev · min⁻¹) for 30 sec. CO₂ production rate was measured with an Ultragas 3 CO₂ analyzer (Wösthoff Company, Bochum, FRG). For determination of basal respiration (mg CO₂ · hour⁻¹ · 100 g⁻¹), nonamended soil samples were allowed to equilibrate for at least 10 hours after connection to the analyzer, and CO₂ evolution was then measured for at least 5 hours.

Carbon

Soil organic carbon (C_{org}) was determined by dry combustion (Leco induction furnace) after expelling carbonate-C by addition of HCl (10%). HCl was added dropwise until effervescence was no longer observed; subsequently the samples were dried at 70°C. Carbonate was calculated from the difference between total carbon (C_{tot}) (dry combustion without HCl addition) and C_{org}.

Calculation and Statistics

The data are arithmetic means of three single measurements (or calculations for the C_{micr}/C_{org} ratio and qCO₂). Regressions and correlations with age as the independent variable were calculated as the “best fit” out of five options ($y = a + bx$, $y = a \cdot e^{bx}$, $y = a + b \cdot \ln x$, $y = a \cdot x^b$, $y = a + b_x + cx^2$).

Corrections for Lignite Carbon

Lignite dust depositions had increased the C-content of several sites. In order to distinguish between lignite carbon and organic carbon from recent plant material, it was necessary to assess the portion

Table 1. Description of the sampling plots

Site ^a	Slope aspect	Vegetation	Particle size distribution ^c (%)			Soil profiles	Fertilizer treatments (kg·ha ⁻¹ a ⁻¹)	Date of sampling
			Sand	Silt	Clay			
F1	±plain	none				IU		1 (=18.12.85)
F2a	5 SO	be/(po)	9.3	71.3	19.7	IU		1
F2b	5 W	be/la,ma	26.3	57.1	16.6	sIU		1
F3a	10 SW	be/li,la				IU		1
F3b	6 W	be/la(po)	17.9	65.6	16.6	IU		1
F4a	26 W	be/(po)				IU		1
F4b	26 W	be/la(po)	81.0	11.3	7.7	IS		1
F4c	26 W	be/(wi,po)	83.6	10.3	6.1	IS		1
F5a	26 W	be/la(po)				IS		1
F5b	26 NW	be/(po)				IS		1
F5c	26 W	be/la	74.7	15.4	9.9	IS		1
F6	26 NW	be/la				IS		1
F17	11 SW	be	86.7	8.0	5.3	IS		1
F21a	Plain	be	27.7	53.5	18.9	uL	O ₁ (cm), O _r 1 (cm)	2 (=11.6.85)
F21b	Plain	be	35.0	47.8	17.2	suL	O ₂ , O _r ²	1 + 2
F22a	12 N	be	82.0	9.3	8.7	IS	O ₂ , O _r ²	1 + 2
F22b	13 N	be	87.7	6.6	5.7	IS	O ₁ , O _r 1	1 + 2
F24	3 NW	be	87.1	8.5	4.4	S	O _b , O _r 1, A _b 3	1 + 2
F25a	Plain	be	83.3	9.1	7.6	IS	O ₁ , O _r 1	1
F25b	Plain	be	79.4	13.3	7.3	IS	O ₁ , O _r 1	1 + 2
F27	7 SO	be				IS	O ₁ , O _r 1	1 + 2
F29	Plain	be	73.5	19.2	7.3	IS	Not determined	1
F30	4 NW	be	28.3	54.7	17.0	sIU/uL	Not determined	1 + 2
F33	Plain	be	22.7	58.0	19.3	uL	O ₁ , O _r 1, O _r 5	1 + 2
F46a	Plain	be	63.8	17.4	18.8	sL	O ₁ , O _r 1, O _r 2, A _b 6	1 + 2
F46b	1 N	be	68.2	15.7	16.8	IS	O ₁ , O _r 1, O _r 4	1 + 2

Generally anthropogenic, no horizons developed on the younger sites (YY-horizon), with only a thick layer of nearly undecomposed herbaceous litter visible. Distinct horizons cannot be distinguished before the 17th year

Table 1. Continued

Site ^a	Slope (%) aspect	Vegetation	Particle size distribution ^c (%)			Soil profiles	Fertilizer treatments (kg ha ⁻¹ a ⁻¹)	Date of sampling
			Sand	Silt	Clay			
A1	± plain	---w ^b					4 (=10.4.86)	
A2	± plain		42.2	43.6	13.3	uIS	3 (=14.11.85)	
A2b	± plain	---ww					4	
A12	± plain		29.5	53.4	17.1	uL	3	
A13	± plain	gggbw					4	
A17	2 SW	ccccc	38.6	29.7	31.8	stL	4	
A18	2 SW	wbwws	18.0	71.0	11.0	lU	4	
A22	± plain	swwsb	45.4	36.4	18.2	sL	4	
A29	± plain	wswsw					3	
A31	2 S		22.3	50.6	27.1	tl	4	
A32	2 S	wswws					3	
A33	2 S	bswbs	47.0	42.0	11.0	uIS	4	
A34	± plain						4	
A35	± plain	wswsb	39.9	36.6	23.5	sL	4	
A41	± plain	swbsw	27.5	44.7	27.8	tl	4	
A57a	± plain	swsw	38.5	42.4	19.1	suL	3	
A57b	± plain						4	

Anthropogenic soil consisting of loess, in some cases mixed with glauconite (tertiary). Ap horizon generally 30 cm

Abbreviations: beech (be), poplar (po), lime (li), willow (wi), larch (la); trees in parentheses were planted only to nurse the commercial species and were eventually replaced by them
^a Numbers give the ages (in years) of the agricultural (A) and forest (F) sites
^b Crops grown on the sites the last 5 years: wheat (w), barley (b), corn (c), sugar beet (s), grass (g)
^c Has not been measured for all soils, in some cases only an estimate of texture has been made. Sand, sandy (S,s), silt, silty (U,u), clay, clayey (T,t), loam, loamy (L,l)

Table 2. Chemical site properties

Site	pH (CaCl ₂)	Carbon- ate C (%)	Lignite- carbon (%)	Nitrogen (%)	C/N
F1	7.2	0.1	—	0.026	11
F2a	7.4	0.2	—	0.026	11
F2b	7.2	0.3	—	0.029	9
F3a	7.3	0.3	—	0.027	11
F3b	7.1	0.1	—	0.031	11
F4a	5.6	—	—	0.017	13
F4b	6.6	—	—	0.021	16
F4c	4.8	—	—	0.015	12
F5a	6.8	0.1	—	0.030	14
F5b	5.8	—	—	0.022	10
F5c	5.4	—	—	0.014	10
F6	4.9	—	—	0.014	16
F17	4.6	—	—	0.078	17
F21a	6.8	—	1.03	0.172	23
F21b	6.2	—	0.32	0.125	20
F22a	4.9	0.1	0.25	0.071	21
F22b	4.9	0.1	0.47	0.054	25
F24	7.1	—	4.17	0.124	44
F25a	6.9	0.1	3.13	0.185	31
F25b	6.9	—	2.32	0.119	33
F27	7.1	—	2.14	0.083	38
F29	5.0	—	0.23	0.083	20
F30	7.1	0.2	3.68	0.157	36
F33	6.6	—	1.41	0.127	27
F46a	6.2	—	8.30	0.218	48
F46b	6.3	0.3	0.98	0.124	25
A1	7.5	0.4	—	0.028	9
A2a	7.2	0.6	—	0.020	10
A2b	7.4	0.4	—	0.034	9
A12	7.1	0.2	0.02	0.048	12
A13	7.5	0.1	0.01	0.037	14
A17	7.4	0.3	—	0.040	11
A18	7.4	0.2	—	0.041	10
A22	7.3	0.1	0.06	0.038	13
A29	7.3	0.1	—	0.075	10
A31	7.3	0.1	0.22	0.062	15
A32	7.3	0.1	0.04	0.068	13
A33	7.4	0.3	0.03	0.061	12
A34	7.4	0.1	0.03	0.053	12
A35	7.5	0.4	0.04	0.063	13
A41	7.4	0.1	0.02	0.063	12
A57a	7.0	—	0.18	0.097	14
A57b	7.4	0.3	0.26	0.075	15

of C_{org} attributable to lignite. The C/N ratio ($C_{\text{org measured}}/N_{\text{total}}$) was used as a calculation base. We assumed that the C/N ratio without lignite would not be more than 12 on the agricultural sites and 18 on the forest sites. For all sites with a C/N ratio higher than 12 or 18, the C_{org} value was corrected, assuming that the C/N ratio of lignite was 75.

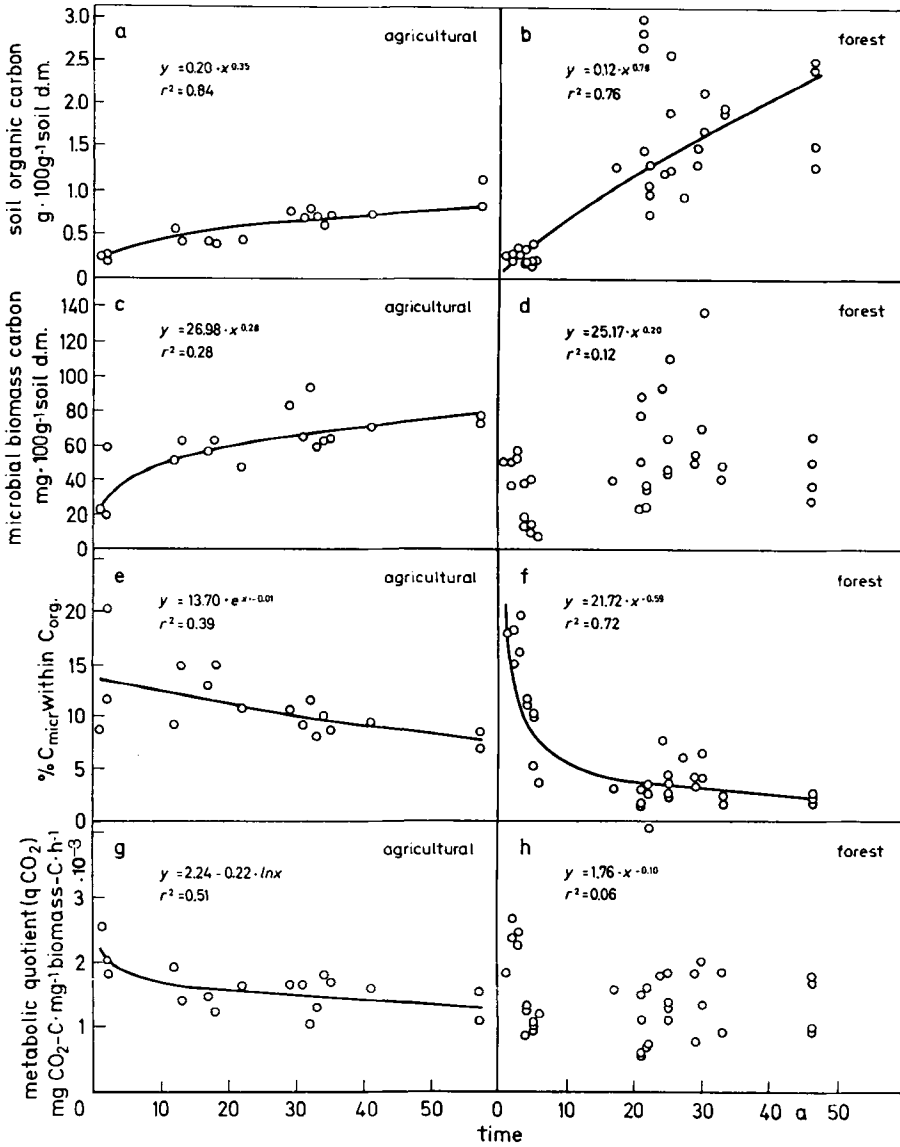


Fig. 1. Relationship between site age and some properties of the soils from the agricultural and the forest chronosequences.

Results and Discussion

Accumulation of organic matter was more rapid on the forest sites (Fig. 1b) than on the agricultural ones (Fig. 1a). On the agricultural sites, soil organic carbon accumulated by far faster during the first years after reclamation than later on. Using the “best fit” regression, the annual carbon accumulation was 0.031% during the first 5 years and 0.011% for the subsequent 40 years. It

would take 90 years to reach a C_{org} level of 1% on the agricultural sites if the curve was extrapolated. A level of 1% is common for agricultural land in temperate regions, if data for organically manured soils are omitted [3, 12]. The high level for one of the 57-year-old sites (A57a) was probably caused by organic manuring during the first 20 years. Burykin [6] (and several authors cited by him) obtained similar results on other reclamation sites, with carbon accumulation rates decreasing with time.

On the forest sites, annual carbon accumulation was 0.059% during the first 5 years and 0.052% over the next 40. It may be concluded from the regression line that equilibrium was not reached within the period investigated (46 years). The C_{org} content of a nearby natural forest stand, however, was not higher than that of some of the reclaimed sites. In contrast to the more or less continuous conditions on the agricultural sites, soil-forming conditions changed drastically as the forest sites developed, primarily due to changes in the structure of the plant cover. On these sites the impact of herbaceous plant residues and root exudates probably decreased with time; the trees themselves would have altered the microclimate as they grew. These results can be compared with those of Dickson and Crocker [7] who investigated a chronosequence in a *Pinus ponderosa* forest and found that it took 500 years for soil organic carbon to reach a steady state equilibrium. Even then a natural forest sequence undergoes changes, e.g., a monospecied canopy diversifies with time.

This comparison of development on agricultural and forest sites must not be pressed too far. Only the top 15 cm layer was sampled on both the agricultural and forest sites. In the agricultural soils C_{org} was approximately the same throughout the A_p -horizon (30 cm) and thus "diluted" with subsoil, whereas on the undisturbed forest sites the carbon content followed a gradient, the layer of the mineral horizon with the highest C_{org} content being sampled. This might have led to an overestimation of C_{org} in the mineral horizon of the forest sites. This would, however, have been counterbalanced by the exclusion of the organic matter in the O horizons (see Table 1).

Microbial biomass recovered quickly on the agricultural sites (Fig. 1c); 15 years after reclamation, the biomass levels had reached $57 \text{ mg} \cdot 100 \text{ g}^{-1}$, which is more than 70% of the 50-year level. These figures are in good agreement with those of old agricultural soils [1]. The rapid recovery of microbial biomass indicated that carbon and energy requirements of soil microorganisms were mainly provided by fresh organic matter. Crop yield (and presumably also the input of available carbon) was not much lower on the young sites than on the older ones. Assuming that the annual carbon input to the soils investigated was $1,000 \text{ kg} \cdot \text{ha}^{-1} \cdot \text{a}^{-1}$ in the 10 cm sampling depth [3], and the bulk density was 1.33, the annual carbon input was 0.075% of soil dry mass. As outlined above, C_{org} increased at a rate of 0.031% annually during the first 5 years. Thus, on average, 60% of the annual carbon input was lost each year, probably through respiration, in good accord with data given by Sauerbeck and Gonzalez [19] and others.

For the forest chronosequence, the correlation between site age and microbial biomass was not significant. Even on the youngest sites, the levels of C_{micro} were comparable to those of mature forest A_h horizons (Table 3) and [15]. However, overall decomposer activity is probably higher in a mature forest floor if changes in spatial distribution of the decomposer community during maturing are al-

Table 3. Properties of a mature beech forest A_h -horizon located near the forest-chronosequence study sites (Rhineland lignite mining district)

Texture	Clay
C_{org}	2.6%
N_{tot}	0.15%
C/N	17.5
C_{micr} (mg · 100 g ⁻¹ soil)	22.2
C_{micr}/C_{org}	0.84%
qCO ₂ (mg CO ₂ -C · h ⁻¹ · mg ⁻¹ C _{micr})	$2.25 \cdot 10^{-3}$
pH (CaCl ₂)	3.8

Table 4. Linear correlation analysis of microbial and pedological properties (correlation coefficient, *r*)

Agricultural chronosequence								
Age	C_{org}	C_{micr}	C_{micr}/C_{org}	N_{tot}	pH	Clay % ^a	n = 17	
C_{org}	0.91	1	0.77 ^c	-0.59 ^c	0.99 ^c	-0.37	0.24	
C_{micr}	0.68 ^c	0.77 ^c	1	-0.04	0.79 ^c	-0.09	0.18	
C_{micr}/C_{org}	-0.55 ^d	-0.59 ^d	-0.04	1	-0.54 ^d	0.31	-0.09	
qCO ₂	-0.44	-0.40	-0.59	-0.23	-0.39	0.12	-0.03	
Forest chronosequence								
Age	C_{org}	C_{micr}	C_{micr}/C_{org}	N_{tot}	pH	Clay % ^b	n = 37	
C_{org}	0.71 ^c	1	0.59 ^c	0.66 ^c	0.92 ^c	0.27	0.27	
C_{micr}	0.30	0.59 ^c	1	0.00	0.63 ^c	0.58 ^c	0.14	
C_{micr}/C_{org}	-0.72 ^c	0.66 ^c	0.00	1	-0.59 ^c	0.18	0.12	
qCO ₂	-0.14	-0.10	0.26	0.36 ^d	-0.06	0.09	0.27	

^a n = 10^b n = 17^c $P < 0.01$; ^d $P < 0.05$; others are not significant

lowed for. On the young sites the contribution of herbaceous roots and litterfall to the carbon input is greater than later on. As the forest develops, primary decomposition shifts more and more from the mineral horizon to the litter layer. Only one-tenth of the forest floor microbial biomass is located in the A_h -horizon in mature deciduous forest [13, 15].

The amount of microbial biomass carbon within the pool of soil organic carbon decreased with time on both the agricultural and the forest sites (Fig. 1e, f). On the agricultural sites the values were unusually high even after 50 years compared to published data which range between 2 and 7% [3, 4, 12]. Beck [5] put forward the hypothesis that soils that exhibit a level of bioactivity per unit C_{org} higher than an empirical average are "developing" soils with a net accumulation of organic carbon, and those with a lower level are losing organic carbon from a pool of relatively stable humic materials. Since the agricultural soils of this study exhibited C_{micr}/C_{org} ratios higher than those commonly found under crop rotations [3], it must be assumed our soils investigated were still in a very early stage of carbon accumulation, in accord with the observed accumulation of carbon (Fig. 1a).

On the forest sites, a very distinct relationship between age and the $C_{micr}/$

C_{org} ratio of the A horizon was observed (Fig. 1f), in contrast to the very poor correlation between microbial biomass content and time (Fig. 1d). Small amounts of C_{micr} within a large pool of C_{org} means that the "average availability" of the carbon source must be low. The rapid decrease of the $C_{\text{micr}}/C_{\text{org}}$ ratio with time (Fig. 1f) means that the availability of carbon (but not the amount) in the A horizon decreased. A progressively reduced carbon availability can be expected as the forest floor stratifies during maturation: those components of the litter that are readily available are utilized by primary decomposers in the O horizon. The more recalcitrant compounds remain and move into the mineral horizon. This is in accord with the data from the old beech forest (Table 3), where microbial biomass was only a very small component of the soil organic matter.

The $q\text{CO}_2$ decreased significantly on the agricultural sites (Fig. 1g). This strongly supports the hypothesis that microbial respiration per unit microbial biomass decreases as the system matures. On the forest sites the decrease of $q\text{CO}_2$ was not significant. It might be that energetic optimization, the principle behind this hypothesis, had not enough selective pressure in the young forest floor, where conditions for microbial growth were permanently undergoing change.

In general, the deviations from the regression lines (Fig. 1) were very high, especially on the forest sites. Obviously, interrelations of C_{org} and C_{micr} with pH and clay content partly mask the influence of age (Table 4). If the values in Fig. 1b and 1d are compared with the data on particle size distribution (Table 1), it can be seen that a high clay content was associated with high C_{org} values (sites F21, F30, F33). High sand content had the opposite effect (e.g., site F46). Generally, the influences, other than site age, were higher for C_{micr} and C_{org} as sole parameters than for the combination of both ($C_{\text{micr}}/C_{\text{org}}$ ratio) (see Table 3).

Lawrey [16] stressed the importance of relating structure of an ecosystem with functions. Several authors [20, 24] have since reported that single properties of reclaimed mine spoils may recover within a few years (enzyme activities, litter decomposition, ATP content). However, it generally takes longer for a steady state to be reached, when the system is considered as a whole.

The same conclusions follow from this study. The best evidence that the systems we investigated had still not reached a steady state after 50 years came from a combination of the two parameters, organic C accumulation and microbial biomass, rather than from either of them taken separately. This general conclusion also confirms earlier botanical [27] and pedozoological investigations [8] on comparable sites.

The data given in this paper suggest that the $C_{\text{micr}}/C_{\text{org}}$ ratio can be used as a measure of the success of reclamation efforts. Measurement of the $q\text{CO}_2$ appears to be of less practical value.

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