

Microbial biomass and earthworm populations in relation to soil chemical parameters in an oak-beech forest soil

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Synopsis

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We studied the influence of liming and P/K fertilization in an oak-beech (*Carpinus betulus-Quercus robur*) forest soil on the microbial biomass and the earthworm population seen in relation to varying soil chemical parameters. Therefore the changes of the pH values as well as the contents of mobile nutrients (Ca, Mg, K, P), heavy metals (Zn, Mn, Cd, Pb) and aluminium in the first three years after the treatments were taken into consideration.

Liming led to decreasing contents of mobile heavy metals and mobile aluminium and increasing contents of the basic cations Ca and Mg in the Ah horizon. The biomass of the microflora and of earthworms increased after the treatment, taking a similar development. The content of mobile nutrients showed positive correlations with the biomass of both microflora and earthworms in the plots treated, whereas mobile heavy metals and aluminium showed negative relationships. The goodness of fit between soil chemical parameters and the biomass of the microflora and of earthworms was dependent on the initial supply with nutrients and potentially harmful substances.

Forest soil, oak-beech-forest, liming, P/K fertilization, nutrients, heavy metals, aluminium, microbial biomass, earthworms

1 Introduction

About 78 % of the German forest soils are strongly to extremely acidic with pH values below 4.2 (VEERHOFF & al. 1996). In acidic forest soils the supply

with mobile basic nutrients such as Ca and Mg is low whereas increasing concentrations of mobile heavy metals and aluminium are found (ULRICH 1981, MAYER 1985). In acidic forest soils the composition of the soil fauna differs from that in soils with a better supply of nutrients (SCHAEFER 1989). The abundance of earthworms is particularly low in acidic soils (AMMER 1992, SCHAEFER 1989), also the microbial biomass and its activity are reduced. Consequently, organic layers with partly undisturbed litter are found on mineral soil. In the past ten years countermeasures such as liming and fertilization in forest soils took place. The effects of these treatments on soil chemical parameters and soil fauna as well as its activity have been described by several authors (SCHAEFER & SCHAUERMANN 1990, GEISSEN & al. 1997, SCHÖNING & al. 1997).

As a basis for our study liming in different quantities partly combined with P/K fertilization was carried out in an acidic oak-beech (*Carpinus betulus-Quercus robur*) forest soil, to investigate the influence on the mobile fraction of basic cations, heavy metals and aluminium as well as on the microflora and the earthworm populations. The study concentrates on the changes of these abiotic and biotic parameters in the first three years after the treatments and describes the relationships between them.

2 Materials and Methods

The study was carried out in an oak-beech forest (*Carpinus betulus-Quercus robur*), about 150 years old, in the Kottenforst near Bonn. The test site is located on a plain with Stagnic Gleysols of loess as typical soils. The humus form can be described as moder humus. In 1988, 1994 and 1995 six plots of 2500 m² in size (A1–A6) were treated with different quantities of

Table 1

Treatments of the plots A1–A6:

Liming in Oct. 1988, Jan. 1994 and Jan. 1995 (t dolomite / ha);

P/K fertilization in June 1994 (200 kg P₂O₅ / ha as acidified rockphosphate, 150 kg K₂O / ha as K₂SO₄).

	A1	A2	A3	A4	A5	A6
1988	0	3	3	3	3	3
1994	0	0	3	3+P/K	6+P/K	6+P/K
1995	0	0	0	0	0	6
total	0	3	6	6+P/K	9+P/K	15+P/K

lime, partly combined with P/K fertilization (Tab. 1). In the years between 1993 and 1996 we studied the effects of the different treatments on soil chemical parameters, the microflora and earthworm populations. The results of plot A5 are not presented because of technical problems during fieldwork.

Sampling took place each year in May and October. The first sampling was carried out in October 1993, three months before different quantities of lime were applied in January 1994. In order to investigate the microbial biomass and the content of the mobile elements in the soil at each sampling date, 12 soil cores with a diameter of 5 cm each were taken per plot and separated into the organic layer (Of), the Ah and the AhSw horizon (0–5 cm, 5–10 cm depth). Each four of the samples obtained from the same depth were mixed to gain three mixed samples in total per depth and plot for laboratory analyses. The pH(CaCl₂) value as well as the mobile fraction of macronutrients (Ca, Mg, K, P), aluminium and heavy metals (Mn, Zn, Pb, Cd) were examined (NH₄NO₃ extraction; ZELEN & BRÜMMER 1991). The microbial biomass was determined by the substrate-induced-respiration method (ANDERSON & DOMSCH 1978). Analyses of all samples were carried out in two replicates.

To study the earthworm populations ten soil cores with a diameter of 25 cm were taken and separated into the organic layer (Of), the Ah (0–5 cm), the AhSw (5–10 cm) and the Sw horizon (10–25 cm) at each date and in each plot. The earthworms were extracted by a combined method of hand sorting, sieving and heat extraction (DUNGER &

FIEDLER 1989) and were then weighed and identified on species level. Pre-tests showed that anecic earthworm species do not live in the plots. Therefore we chose the method described to investigate the epigeic and endogeic species. Here we only present the results of the Of and Ah horizon because 95 % of the individuals were found in these depths.

For statistical analyses the H- and U-test and the Spearman correlation were used. The H-test significance was based on a level of $p < 0.05$ and the U-test probability was corrected following the SIDAK procedure.

3 Results

3.1 Soil chemical parameters

Even though the plots A2 to A6 received the same amount of dolomite in 1988 and showed the same vegetation and general soil conditions, the initial supply with nutrients was higher in A4 in 1993 than in the other plots. In all plots treated in 1994 liming led to increasing pH values in the Ah horizon (plots A3, A4, A6). At the same time the content of mobile nutrients such as Ca and Mg increased while the relatively high contents of mobile heavy metals (Mn, Zn, Pb, Cd) and aluminium decreased (Fig. 1, Tab. 2). Due to that fact the contents of heavy metals in the plots treated in 1994 decreased below the trigger values for toxic effects on plants mentioned in the soil protection law of Baden-Württemberg (UMWELT MINISTERIUM BADEN-WÜRTTEMBERG 1993).

Table 2
Content of mobile nutrients (K, P) and mobile heavy metals (Mn, Cd, Pb) in the Ah horizon (0–5 cm) of the different plots

in October 1993 (O93) and May 1996 (M96); (\bar{x} : mean, s: standard deviation; values in mg kg⁻¹).

plot	K				P				Mn			
	O93		M96		O93		M96		O93		M96	
	\bar{x}	s	\bar{x}	s	\bar{x}	s	\bar{x}	s	\bar{x}	s	\bar{x}	s
A1 (0+0)	102.9	0.7	129.2	26	2.23	0.02	3.62	0.30	29.8	0.8	113.5	47
A2 (3+0)	78.5	2.1	95.1	9	0.65	0.08	1.17	0.07	36.7	0.2	66.4	13
A3 (3+3)	63.4	0.3	116.8	27	0.67	0.02	0.62	0.10	43.9	0.2	17.1	5.5
A4 (3+3+P/K)	132.5	1.9	240.8	44	1.17	0.09	1.20	0.50	35.3	0.3	12.1	4.3
A6 (3+12+P/K)	70.2	1.1	151.9	19	0.74	0.02	0.77	0.14	53.9	0.2	6.1	1.8

plot	Cd				Pb			
	O93		M96		O93		M96	
	\bar{x}	s	\bar{x}	s	\bar{x}	s	\bar{x}	s
A1 (0+0)	0.12	<0.01	0.17	0.03	7.74	0.20	4.33	1.0
A2 (3+0)	0.04	<0.01	0.05	0.01	0.85	0.07	0.58	0.3
A3 (3+3)	0.13	<0.01	0.01	0.01	2.89	0.05	0.06	0.1
A4 (3+3+P/K)	0.06	<0.01	0.01	0.00	0.59	0.02	0.01	0.0
A6 (3+12+P/K)	0.05	<0.01	0.01	0.01	1.87	0.05	0.00	0.4

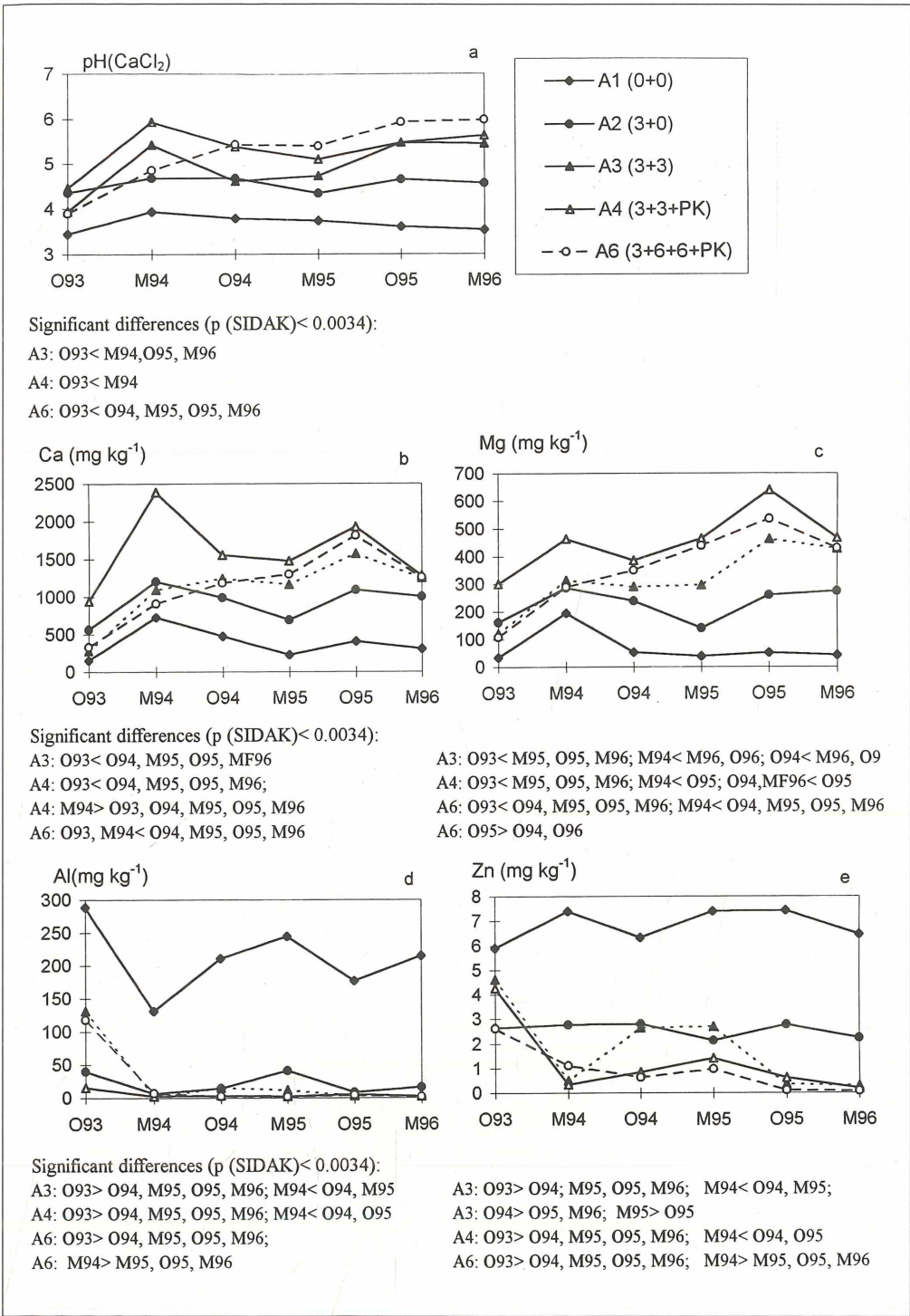


Fig. 1a–e

Changes in the pH(CaCl₂) values (a) and the content of mobile Ca (b), Mg (c), Al (d) and Zn (e) in the Ah horizon (0–5 cm) of the

different plots from October 1993 (O93) to May 1996 (M96); (values in mg kg⁻¹).

3.2 Microbial biomass

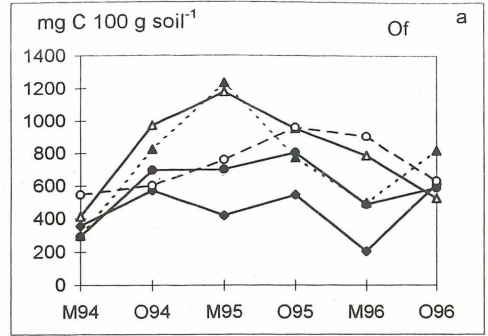
The microbial biomass was strongly influenced by climatic conditions during the seasons as is shown by the variations over the time in the plots A1 and A2 untreated in 1994 (Fig. 2). Therefore we compared the microbial biomass of the samplings in May and of those in October separately using the U-test. Liming with 3+3 t dolomite / ha led to a short term increase in the microbial biomass (A3, A4) in the Of horizon (Fig. 2 a) reaching a maximum in May 1995 (A3, A4). In A6 (3+6+6+P/K) the biomass increased more slowly and reached its maximum at a later date than the other plots, i.e. in October 1995 and May 1996 due to the fact that a third treatment was carried out in this plot in January 1995. In October 1996 a significant effect of the treatments on the microbial biomass could no longer be found in any plot. The microbial biomass in all plots decreased to the level of May 1994.

In the Ah horizon (Fig. 2 b) the overall microbial biomass was about ten times lower than that in the Of horizon because of a much lower content of organic matter. In all plots treated in 1994 the microbial biomass increased on a short term basis in October 1994. In the plots A3 (3+3) and A6 (3+6+6+P/K) this effect was significant, whereas it was not significant in A4 (3+3+P/K) which already had a higher initial microbial biomass in October 1993 (Fig. 2 b). A6 was the only plot to show a continuing effect of the treatment until October 1996.

3.3 Earthworm populations

Three types of earthworms were found in the plots A1 to A6: epigeic species (*Dendrobaena octaedra*, *Dendrobaena pygmaea*, *Dendrobaena rubida rubida*), endogeic species (*Aporrectodea caliginosa*, *Aporrectodea limicola*, *Aporrectodea rosea f. interposita*) and the intermediate species *Lumbricus rubellus rubellus*. Before the second liming took place in October 1993 both, biomass and abundances of earthworms were quite low in the plots A1, A2, A3 and A6 with a biomass < 5 g m⁻² and abundances < 30 individuals m⁻² (Fig. 3 a, b). Even though plot A4 was treated in the same way in 1988 as the other plots, the abundance (71 individuals m⁻²) and biomass (13 g m⁻²) of earthworms were higher in this plot in October 1993, as it is also shown for the microbial biomass in the Ah horizon (see Fig. 2 b).

The plots A1 and A2 untreated in 1994 were used as a reference, because variation of the earthworm populations in these plots was only subject to natural fluctuations. In all plots treated in 1994 liming led to an increase in biomass and abundance of earthworms (Fig. 3a, b). The earthworm populations



Significant differences ($p(\text{SIDAK}) < 0.0069$):

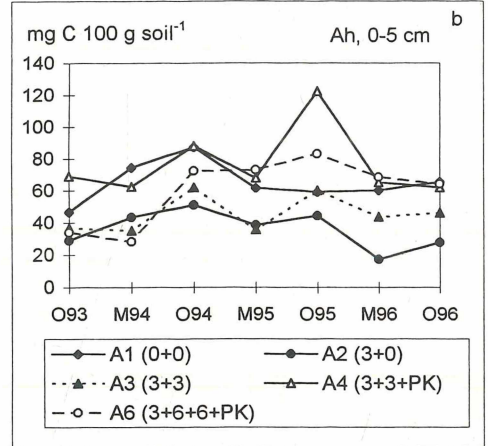
A1: M96 < M94, M95

A2: M95 > M94, M96

A3: M95 > M94, M96

A4: M95 > M94, M96; O96 < O94, O95

A6: O95 > O94, O96; O94 < O96



Significant differences ($p(\text{SIDAK}) < 0.0069$):

A1: O93 < O94, O96

A2: O93, O96 < O94, O95; M96 < M95

A3: O93 < O94, O95

A4: O95 > O93, O94, O95, O96

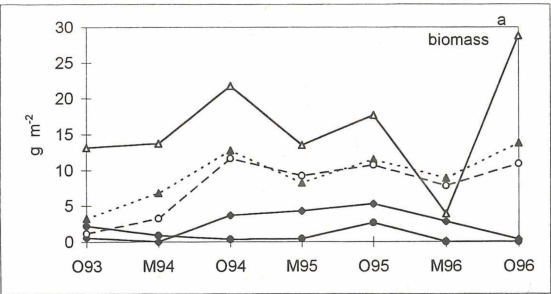
A6: O93 < O94, O95, O96; M94 < M95, M96

Fig. 2 a, b

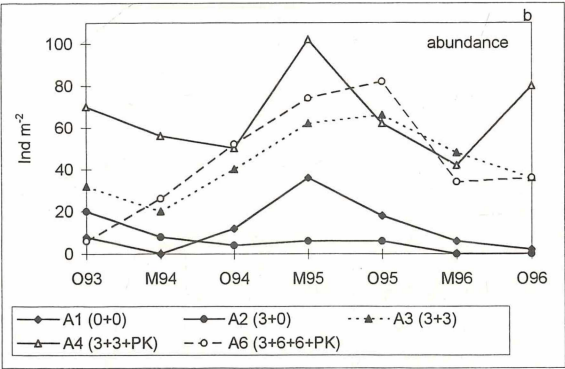
Changes in the microbial biomass in the Of (a) and Ah horizon (0-5 cm) (b) of the different plots from October 1993 / Mai 1994 (M94) to October 1996 (O96); (values in mg C 100 g soil⁻¹); significant differences of the microbial biomass between the sampling dates (no sampling in October 1993 in the Of horizon).

took a similar development in A3 (3+3) and A6 (3+6+6+P/K) over the period of investigation. The abundances in these plots reached a maximum in October 95, whereas the biomass increased from October 1993 to October 1994 and then remained quite constant from October 1994 to October 1996. This is due to the fact that the number of juvenile individuals strongly increased shortly after the treatment. In the plot A4 (3+3+P/K) liming and fertilization also triggered a short term increase in the earthworm population which was, however, less obvious than the effects brought about in the other plots. This again can be put down to a higher initial abundance (Fig. 3 b).

Taking a look at the dominances of the species in the different plots over the three years of investigation we found that in the plots A3 and A6 a definite increase in the dominances of the endogeic species *Aporrectodea* ssp. took place after liming in January 1994 (Tab. 3). That means that the increase of the earthworm populations is caused by an increase of the endogeic *Aporrectodea* ssp. In A4 the dominances of *Aporrectodea* ssp. remained quite constant during the period of investigation. In the plots A1 and A2 untreated in 1994 the dominances of the endogeic species remained generally low.



Significant differences (p (SIDAK) < 0.0034):
A2: O93>M94, O94, M95, M96, O96
A4: M96<M95, O94, O96
A6: O93<O94, M95, O95, M96, O96



Significant differences (p (SIDAK) < 0.0034):
A1: M95>M94, M96, O96
A2: O93>M94, O94, M95, M96, O96
A4: M96 < O94, M95, O96
A6: O93<O94, M95, O95, M96, O96; O95>M96, O96

Fig. 3 a, b
Changes in the total biomass (a) and the total abundances (b) of all earthworms in the different plots from October 1993 (O93) to October 1996 (O96), (values in g m⁻²; individuals m⁻²); (M=May); significant differences of the biomass (a) and abundances (b) between the sampling dates.

Table 3

Dominances of the earthworm genera *Lumbricus* ssp., *Dendrobaena* ssp. and *Aporrectodea* ssp. in the different plots from October 1993 (O93) to October 1996 (O96) (values in %; M=May); abundances of all individuals (values in ind. m⁻²).

A1 (0+0)							
genus	O93	M94	O94	M95	O95	M96	O96
Lumbricus	0	0	17	50	44	66	0
Dendrobaena	100	0	88	39	44	0	100
Aporrectodea	0	0	0	11	12	33	0
Ind m ⁻²	8	0	12	36	18	6	2
A2 (3+0)							
genus	O93	M94	O94	M95	O95	M96	O96
Lumbricus	0	0	17	67	0	0	0
Dendrobaena	80	100	100	0	0	0	0
Aporrectodea	20	0	0	33	100	0	0
Ind m ⁻²	20	8	4	6	6	0	0
A3 (3+3)							
genus	O93	M94	O94	M95	O95	M96	O96
Lumbricus	0	0	5	9	12	0	0
Dendrobaena	50	10	20	4	24	5	12
Aporrectodea	50	90	75	87	64	95	88
Ind m ⁻²	28	8	40	64	66	48	36
A4 (3+3+P/K)							
genus	O93	M94	O94	M95	O95	M96	O96
Lumbricus	20	22	24	19	16	5	22
Dendrobaena	11	18	36	49	22	25	10
Aporrectodea	59	60	40	32	52	70	67
Ind m ⁻²	71	56	50	102	62	42	80
A6 (3+6+6+P/K)							
genus	O93	M94	O94	M95	O95	M96	O96
Lumbricus	50	0	12	7	2	6	5
Dendrobaena	50	69	11	5	17	6	17
Aporrectodea	0	31	77	88	81	88	77
Ind m ⁻²	8	26	53	80	81	34	36

3.4 Correlations between soil chemical parameters, microbial biomass and earthworm populations

The plots A3, A4 and A6 which were limed in 1994 are selected to describe the correlations between the changes in the content of mobile elements, microbial biomass and biomass of the earthworms in the period from October 1993 to May 1996 (Fig. 4, Fig. 5, Tab. 4 a–c). In all three plots the biomass of the microflo-

ra and the earthworms correlated positively with each other and showed a similar development during the period of investigation. The content of Ca and Mg correlated positively with the microbial biomass as well as with the biomass of all earthworms and the biomass of the *Aporrectodea* species. Mobile Al and heavy metals correlated negatively with the biomass of both, microflora and earthworms. P correlated positively with the biotic biomasses in the plots A4 and A6, treated additionally with P and K. The strongest correlations between all parameters were found in

Tab. 4 a – c

Correlations between soil chemical parameters (the content of mobile nutrients, heavy metals, aluminium) the microbial biomass (mic. biom) and the biomass of all earthworms (L. biom) and the biomass of *Aporrectodea* species (Aporr. biom.) in the Ah horizon of plot

a) A3 (3+3 t dolomite / ha)
b) A4 (3+3 t dolomite / ha + P/K)
c) A6 (3+6+6 t dolomite / ha + P/K)
[Spearman coefficients; * sign. (p<0.05); ** sign. (p<0.01); (n=7)]

a)									
A3 (3+3)									
	mic. biom.	pH	Ca	Mg	Zn	Mn	Pb	Cd	Al
mic. biom			0.65						
L. biom.	0.68	0.79	0.94**	0.83*	-0.89*	-0.54	-0.77	-0.55	-0.60
Aporr. biom.	0.40		0.50		-0.60				
b)									
A4 (3+3+P/K)									
	mic. biom.	Ca	P						
mic. biom.			0.77						
L. biom.	0.57	0.66							
Aporr. biom.	0.43	0.77	0.77						
c)									
A6 (3+6+6+P/K)									
	mic. biom.	pH	Ca	Mg	P	Zn	Cd	Pb	Al
mic. biom			0.88*	0.88*	0.94**	-0.54	-0.60	-0.54	-0.54
L. biom.	0.75	0.64	0.94**	0.94**	0.88*	-0.60	-0.71	-0.65	-0.60
Aporr. biom.	0.89*		0.88*	0.88*	0.94**	-0.54	-0.60	-0.60	-0.54

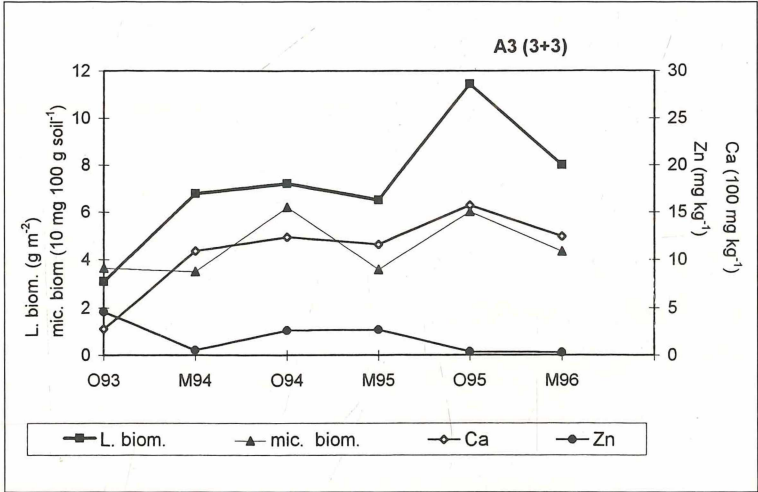


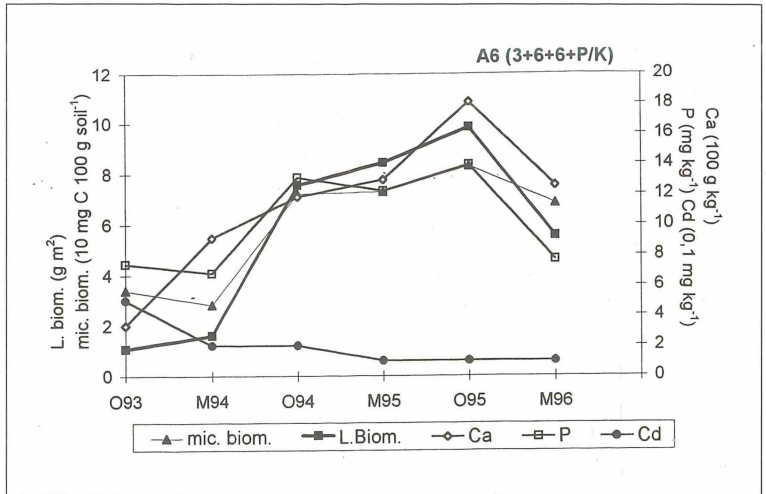
Fig. 4
Changes in the content of mobile Ca (100 mg kg⁻¹), Zn (mg kg⁻¹), the microbial biomass (Mic. biom.) (10 mg C 100 g soil⁻¹) and the biomass of the earthworms (L. biom.) (g m⁻²) in plot A3 (3+3) from October 1993 (O93) to Mai 1996 (M96) in the Ah horizon.

plot A6 (3+6+6+P/K) , which underwent the most intensive changes, the weakest were found in A4 (3+3+P/K) which had better initial conditions. Nevertheless, we confirm that irrespective of the amount of lime applied there is a positive relationship be-

tween the microflora and earthworm population which are both positively influenced by mobile basic cations and negatively influenced by mobile Al and heavy metals.

Fig. 5

Changes of the content of mobile Ca (100 mg kg^{-1}), P (mg kg^{-1}), Cd (0.1 mg kg^{-1}), the microbial biomass (mic. biom.) ($10 \text{ mg C } 100 \text{ g soil}^{-1}$) and the biomass of the earthworms (L. biom.) (g m^{-2}) in plot A6 (3+6+6+P/K) from October 1993 (O93) to Mai 1996 (M96) in the Ah horizon.



4 Discussion

Liming led to a short term increase in the microbial biomass, in particular in the Of horizon. Three years after the treatment in the Of horizon no effect could be seen any more. KREUTZER & ZELLES (1986) also found a short term increase of microorganism populations after liming. The microflora then decomposes the organic substances which are easily available. When this material is decomposed the microbial population is reduced again (KREUTZER 1986).

Our results show positive relationships in the Ah horizon between the supply of available nutrients and the microbial biomass, whereas mobile heavy metals and Al have a negative influence on the microbial biomass and the biomass of earthworms. This corresponds with investigations of BÄTH (1989), CHANDER & al. (1995) and KANDELER & al. (1996) who describe a reduction of activity and biomass of the microflora with increasing contents of heavy metals. It is difficult to compare the trigger values for beginning toxicity and thus decreasing reproduction of soil microflora described by several authors (BÄTH 1989), because they depend on many factors such as content of organic matter and pH value. Furthermore, different methods are used to estimate these trigger values. In most cases the total contents of heavy metals are analysed (STADELMANN & al. 1984). However, a reduction in the pH value of the soil increases the toxicity of heavy metals (TYLER 1981), because the mobile fraction of heavy metals increases in acidic soils (HORNBERG & BRÜMMER 1993). This fraction of available heavy metals in soils plays the most important role for the microflora (STADELMANN & al. 1984). We also suggest to choose the contents of mobile elements as a measure for the effects on biota, especially in acidic forest

soils, where we often find low total contents of heavy metals but high mobile contents. Furthermore, it is worth mentioning that the co-occurrence of different mobile heavy metals – each in concentrations below the trigger values – may lead to a reduced activity and biomass of the microflora (WILKE 1988).

Liming of forest soils led to increasing abundances of earthworms. This is confirmed by investigations carried out by SCHAUERMANN (1985) and MAKESCHIN (1991). Positive correlations were found between the contents of the mobile basic cations Ca and Mg and earthworm populations, heavy metals and Al showed negative effects. Increasing contents of mobile Ca and Mg as a consequence of liming have positive effects on earthworm populations (AMMER 1992, BRIONES & al. 1995), whereas Al and Zn have a negative influence (SPURGEON & al. 1997). Furthermore, the ratio between basic cations and heavy metals has to be taken into consideration, because of the competition between essential and nonessential elements (TYLER 1981).

Changes in soil chemical parameters produce similar changes in the microflora and earthworm populations. The similar development of both can additionally be explained by priming effects. They play an important role in the relationship between soil flora and earthworms (WINDING & al. 1997). Liming increases the proportion of soil bacteria on the microbial biomass which improves the food supply for earthworms (AMMER 1992). Thus earthworms and microflora influence each other in a positive way, which again leads to similar changes in the two biotic parameters.

We conclude that it is the combination of mobile nutrient cations, Al and heavy metals that influences the microflora and the earthworm population, especially in the case of endogeic species which

MAKESCHIN (1993) also described as being strongly dependent on the soil chemical situation.

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