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The effect of lime on microbial activity in spruce (*Picea abies L.*) forests

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Summary. The use of lime increased heat output and decreased the C:N ratio (global indicators of biological activity) in the organic horizons of a spruce forest (*Picea abies L.*) soil. These changes were still present after 18 years. During the same period, the muramic acid content increased slightly, while the concentration of both ergosterol and glucosamine decreased. The ratios of ergosterol or glucosamine to muramic acid decreased significantly after 3 years in the plots that had been irrigated and limed, and after 8 years in the limed non-irrigated plots.

Key words: Muramic acid – Ergosterol – Glucosamine – Soil – Bioactivity – Lime – *Picea abies*

The use of lime in soils normally causes a significant increase in pH and thus a change in soil microbial activity. Nitrification is increased (Bååth et al. 1980; Lang and Beese 1985), carbon exchange increased (Lohm et al. 1984) and the number of bacteria and actinomycetes is increased (Mai and Fiedler 1979; Lang and Beese 1985). However, Bååth et al. (1980) found a strong decrease in the number of fungi and bacteria after the use of lime in a spruce forest, despite an increase in pH. In this paper the effect of lime on the activity of microorganisms is reported (1) in a forest soil which was artificially acidified (short-term effects), and (2) in soils which had been limed at various times in the past (long-term effects).

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Materials and methods

The soil samples for the analysis of the short-term effects of liming were obtained from an 80-year-old (*Picea abies L.*) forest in the Höglwald near Augsburg, Federal Republik of Germany. The soil is an acidic, Parabrown Podzol, with weak pseudogley mottling in the mineral horizon. The treatment of the different experimental plots (each ca. 2500 m^2) has been described in detail elsewhere (Kreutzer and Bittersohl 1986). The following plots were established: Control; watered with acid "rain" (pH 2.7–2.8), watered with "normal" rain (pH 5.0), limed in April 1984 with 4 t ha^{-1} ground dolomite; watered with acid "rain" and limed; watered with "normal" rain and limed. During the warmer periods of the year, the appropriate plots were artificially irrigated, beginning in 1985, with a circular sprinkler system. Each year there were 15–18 "precipitation events" of 10–12 mm "rainfall" over a 70-min period. About 3–4 kmol of protons per hectare were distributed each year on the plots exposed to acidic irrigation.

For the long-term effect of the liming study, not only the Höglwald but also three other regions were used as well (Table 1). The year in which the lime was applied is given in Table 1.

In November 1986 samples were taken with a 10-×10-cm box corer. Each horizon was sampled separately, with five subsamples being taken from each horizon and then mixed to give a representative sample for that horizon. In the Höglwald, five horizons (Of1, Of2, Oh, Aeh, and Ahl) were sampled but only three (Lof, Oh, and Ah) were sampled from the other regions. The samples were immediately sieved and stored at 4 °C.

The C:N ratios were determined on air-dried samples with a Carlo Erba Element Analyzer (model 1106). Dry weight was defined as the weight of the soil after being heated for 24 h at 75 °C. The pH was measured with a glass electrode (WTW pH Digi 510) in a soil:water (1:2) slurry, which had been stirred for 2 h. The heat output (Zelles et al. 1987a) of 1 g fresh soil was measured in a microcalorimeter (LKB 2080).

The equivalent of 1 g dry weight of fresh soil material was hydrolyzed with methanol and then extracted with petroleum benzene. The ergosterol content in the liquid phase was determined with high performance liquid chromatography (Zelles et al. 1987b). For quantitative determinations of muramic acid and glucosamine, 1 g dry weight equivalent of fresh soil was hydrolyzed at 105 °C for 3 h in 10 ml of 6 *N* HCl. From the filtered hydrolysate, 300 µl was removed and dried at 45 °C in a rotary evaporator. The residue was derived for 2 min with 500 µl of an ophthalaldehyde-2-mercaptoethanol solution. The resulting derivative was quantified with high performance liquid chromatography (Zelles 1988). Each measurement was

made in triplicate and the values are given per gram dry weight. The analytical standard deviation is shown in the figures and tables.

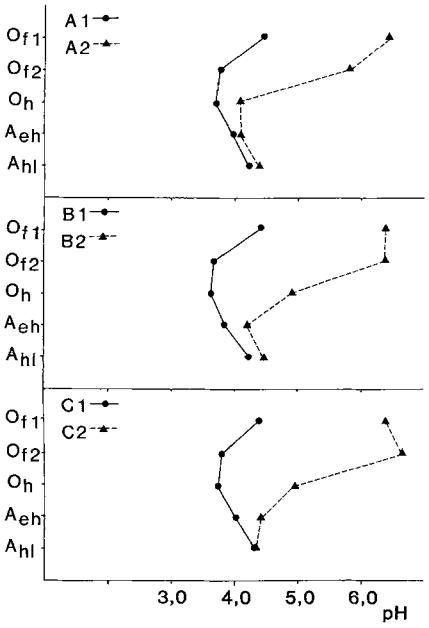

Fig. 1. pH values in organic (Of1, Of2, and Oh) and mineral (Aeh and Ahl) layers of soil of Höglwald. A1 is control, A2 is similar to A1 but limed, B1 is acid irrigated, B2 is similar to B1 but limed, C1 is irrigated, and C2 is irrigated and limed

Results

Short-term effects

The use of lime had a significant effect on pH, mainly in the humus horizons (Fig. 1). Irrigation, with or without the addition of acid, had little effect on the pH in the deeper horizons. The heat output (Table 2) was increased through liming (all limed treatments) by a factor of 2–3 in the Of1 and Of2 horizons, while irrigation had a lesser effect. The addition of protons to the irrigation water suppressed the development of heat in the Of1 and Of2 horizons, and in conjunction with lime also in the Of2 and Oh horizons. Lime did not change the C:N ratio in the non-acidified, non-irrigated plots. Irrigation enhanced the C:N ratio in the Of1 and Of2 horizons while the presence of lime in the irrigated plots decreased it. The ergosterol concentrations (Table 2) were highest in the Of1 horizon in all cases and decreased with depth. Irrigation increased the ergosterol concentrations in the Of1 and Of2 horizons. The glucosamine concentrations were clearly higher in the organic horizons than in the mineral horizons, with a less pronounced increase as a function of depth. Irrigation, with or without the addition of acid, tended to increase the glucosamine concentration in the Of2 horizon. The muramic acid concentration increased after the addition of lime in the Of1 ho-

Table 1. Soil characteristics and treatments

Soil no.	FAO class	Amount (t ha^{-1}) time of liming	Horizons	pH (H_2O)	Total C (%)	Total N (%)	C:N	Thickness (cm)
I	Orthic luvisol	Control	LOf	4.5	48.0	1.78	26.9	3.1
			Oh	3.7	20.5	0.90	22.7	1.6
			Ah	4.0	4.3	0.21	20.4	5.0
		4.0	LOf	6.5	45.4	1.70	26.7	3.1
		55% $CaCO_3$ + 40% $MgCO_3$	Oh	4.1	17.3	0.79	21.9	1.6
		1984	Ah	4.1	4.0	0.20	20.0	5.0
II	Spodic dystric cambisol	Control	LOf	3.9	46.0	1.69	27.2	3.0
			Oh	3.9	39.6	1.41	28.1	4.0
			Ah	4.0	5.5	0.25	21.8	5.0
		2.7	LOf	5.2	44.7	1.79	25.0	3.0
		$CaCO_3$	Oh	3.9	43.1	1.58	27.3	4.0
		1981	Ah	4.0	6.2	0.29	21.3	5.0
III	Spodic dystric cambisol	Control	LOf	4.1	47.9	1.66	29.0	3.0
			Oh	3.6	41.1	1.27	32.4	4.5
			Ah	3.9	4.1	0.15	27.5	5.0
		2.0	LOf	5.4	39.8	1.44	27.6	3.0
		$CaCO_3$	Oh	4.7	29.4	0.94	31.8	4.0
		1978	Ah	4.3	2.8	0.11	25.5	5.0
IV	Spodic dystric cambisol	Control	LOf	3.6	46.5	1.58	29.4	2.5
			Oh	3.4	34.4	1.18	29.2	2.0
			Ah	3.5	4.6	0.17	26.8	5.0
		3.0	LOf	4.8	30.5	1.13	26.9	2.0
		$CaCO_3$	Oh	4.7	17.4	0.69	25.2	2.0
		1968	Ah	5.8	5.2	0.22	23.8	5.0

rizon. Irrigation without liming, irrespective of the proton content, suppressed the accumulation of muramic acid in both the Of1 and Of2 horizons. There were no detectable differences in the ergosterol: muramic acid and the glucosamine: muramic acid ratios (Figs. 2 and 3) due to liming in the non-irrigated plots, but these ratios were lower in the Of horizons of the irrigated and limed plots than in the non-limed, irrigated plots. The differences, however, disappeared with increasing depth.

Long-term effects

Lime enhanced the pH significantly in the upper layer (LOf) when it had been applied comparatively recently (1984, 1981), and in the mineral layer when sufficient

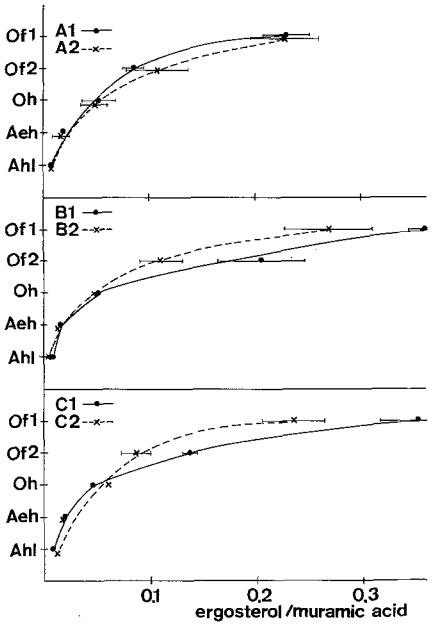

Fig. 2. Ergosterol: muramic acid ratios in organic (Of1, Of2, and Oh) and mineral (Aeh and Ahl) layers of soil of Höglwald. For explanation of treatments, see Fig. 1

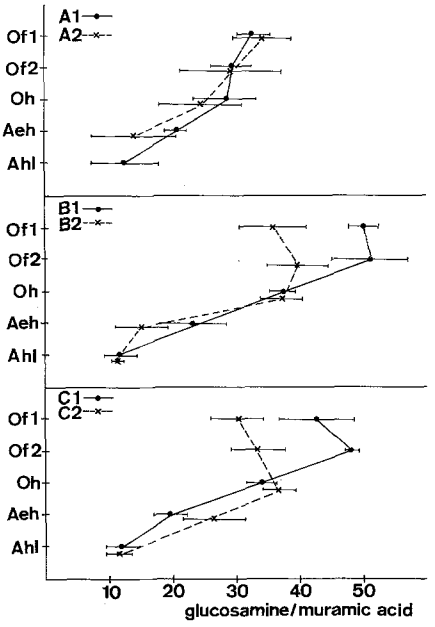

Fig. 3. Glucosamine: muramic acid ratios in organic (Of1, Of2, and Oh) and mineral (Aeh and Ahl) layers of soil of Höglwald. For explanation of treatments, see Fig. 1

Table 2. Heat output ($\mu W\,g^{-1}$ dry weight), C:N ratio, contents of ergosterol, glucosamine and muramic acid ($\mu g\,g^{-1}$ dry weight) in organic (Of1, Of2, and Oh) and in mineral (Aeh and Ahl) horizons

	Heat output		C:N		Ergosterol		Glucosamine		Muramic acid	
	A1	A2	A1	A2	A1	A2	A1	A2	A1	A2
Of1	1024±28	2757±110	27.8	27.4	24.1±1.4	27.1±2.6	3557±143	4206±369	108.1±8.2	122.6±13.4
Of2	291±12	722±8	26.2	26.0	9.7±1.0	12.5±0.4	3488±362	3557±116	117.4±5.1	119.5±16.5
Oh	121±6	102±4	22.4	21.6	3.6±0.0	3.8±1.0	2052±771	2052±567	72.1±22.6	84.5±20.6
Aeh	26±2	31±2	20.2	17.1	0.6±0.0	0.5±0.0	684±55	479±48	31.9±4.1	34.0±4.1
Ahl	6±1	9±0	11.1	8.7	0.1±0.0	0.1±0.0	205±61	479±48	17.5±6.1	17.5±5.1
	B1	B2	B1	B2	B1	B2	B1	B2	B1	B2
Of1	1194±55	3773±101	29.0	26.2	28.1±1.1	26.8±3.1	3830±150	3762±369	77.3±2.0	104.0±11.3
Of2	414±24	1367±73	27.6	24.9	16.1±0.6	11.6±0.7	4172±1224	4377±369	81.4±8.2	109.2±11.3
Oh	157±7	211±7	22.5	22.1	4.3±0.2	3.5±0.3	3215±82	2941±198	86.3±4.1	78.3±4.1
Aeh	29±1	23±0	15.0	17.1	0.6±0.2	0.4±0.1	889±314	479±123	38.1±7.2	28.9±3.0
Ahl	13±1	12±0	10.5	8.0	0.1±0.0	0.1±0.0	205±27	137±7	27.8±4.1	11.3±1.0
	C1	C2	C1	C2	C1	C2	C1	C2	C1	C2
Of1	1785±69	3266±78	28.4	26.7	28.2±1.1	23.5±2.5	3488±355	3146±410	82.4±8.2	103.0±6.1
Of2	520±6	1911±44	27.2	24.5	12.6±0.5	13.5±0.9	3899±68	4377±588	82.4±0.0	131.8±13.4
Oh	108±2	275±2	22.0	22.0	2.7±0.1	5.1±0.1	2120±143	3215±184	61.8±7.2	87.5±5.1
Aeh	34±3	38±0	16.3	14.9	0.6±0.1	0.5±0.0	616±68	547±82	31.9±3.0	26.8±4.1
Ahl	18±1	18±0	10.7	11.9	0.1±0.0	0.1±0.0	205±34	137±20	17.5±2.0	13.4±2.0

Treatments: A1, control; A2, limed; B1, acid precipitated; B2, acid precipitated and limed; C1, irrigated with "normal water"; C2, irrigated with "normal water" and limed

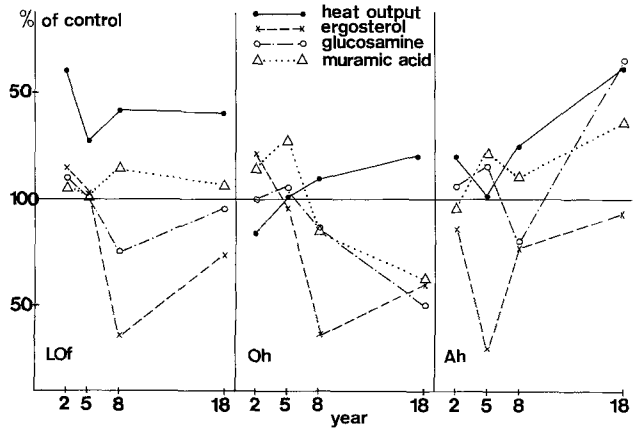

Fig. 4. Changes in various parameters of three soil horizons as a function of time elapsed since experimental plots were limed. All results given as percentage of values obtained for parameters in non-limed plots

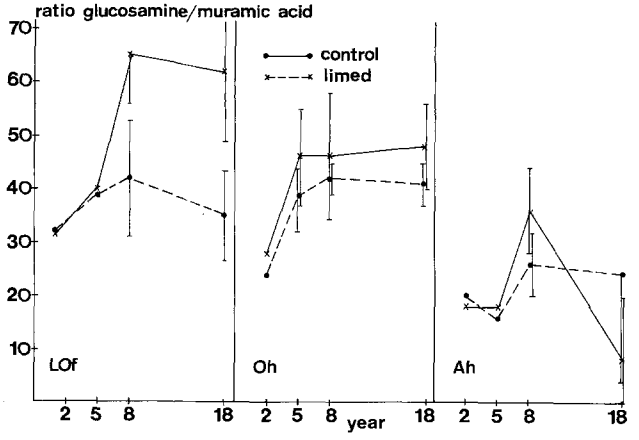

Fig. 5. Glucosamine: muramic acid ratios in three soil horizons as a function of time elapsed since liming of experimental plots

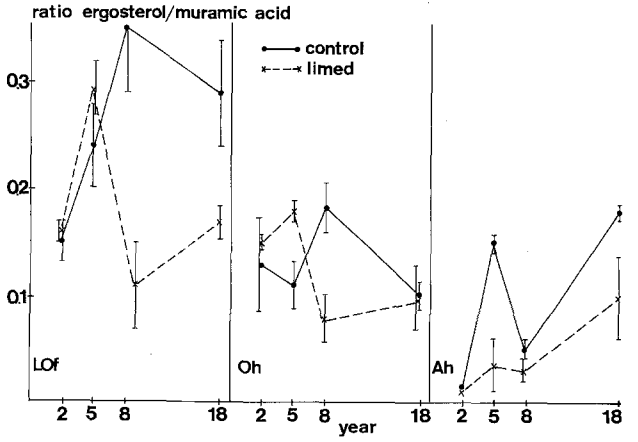

Fig. 6. Ergosterol: muramic acid ratios in three soil horizons as a function of time elapsed since liming of experimental plots

time had elapsed since the application. The C:N ratios were smaller in the latter case (Table 1). After 2 years, the heat output in the LOf horizon of the limed plots was 60% higher than in the non-limed plots, and even after 18 years the activity was still 40% higher than in the controls (Fig. 4). The maximum muramic acid content was moderate after 8 years and then tended to approach the control values (Fig. 4). The ergosterol and glucosamine concentrations decreased as a result of the added lime but recovered after 8 years. In the Oh horizon there was a steady decrease in both ergosterol and glucosamine concentrations and also in muramic acid, even though the heat output tended to increase steadily. In the Ah horizon a strong increase in the heat output and in the concentration of ergosterol and muramic acid was evident after 18 years. The glucosamine: muramic acid ratio (Fig. 5) and the ergosterol: muramic acid ratio (Fig. 6) were definitely lower in the LOf horizon of the limed plots after 8 and 18 years. In the other horizons this trend was considerably weaker or absent.

Discussion

The enhanced heat output shows that liming produces a global increase in microbiological activity. This finding was indicated in previous work where liming increased the production of CO_2 and the content of ATP in soils (Zelles et al. 1987a). Furthermore, in the present study, the pH increased and the C:N ratios became smaller. However, the results obtained in the present short-term experiments show that liming had a negative effect on the ratios of glucosamine and of ergosterol to muramic acid in the Of1 and Of2 horizons when the plots were irrigated. Irrigation can thus be viewed as a catalyst of the microbial processes. In the absence of irrigation, in present long-term experiments, at least 8 years elapsed before the Of horizon showed comparable ratios. In the same set of experiments, an increase in the heat output was also seen in the deeper layers as the lime began to reach them. However, in the Oh horizon (which is most likely to accumulate added lime) this heat production was presumably caused by a relatively few lime-resistant organisms, since all the other measured parameters decreased there. This was not the case in the Ah horizon, presumably because the full impact of liming is not yet present. As with the short-term experiments, liming had a negative effect on the ratios of glucosamine and of ergosterol to muramic acid. The ergosterol: muramic acid ratio was less stable than the glucosamine: muramic acid ratio, most likely because of the higher concentration of glucosamine in non-living material.

These results may mean that fungi are unable to function optimally at a higher pH and that as the acidic habitat changes their ecological role is taken over by the prokaryotes. After liming, the increase in the pH allows a wide range of prokaryotes to develop, and consequently the spectrum of fungal species will be limited. Other possibilities that may explain the results obtained here are that Ca^{2+} directly or indirectly inhibits fungi or that the selected parameters are not an optimal indicator of fungal vs bacterial activity.

The suitability of glucosamine and ergosterol as indicators of fungal activity has been evaluated in different ways in the literature. Seitz et al. (1979) demonstrated that both parameters can indicate the invasion of fungi in milled rice. Grant and West (1986) reported that ergosterol is an indicator of fungal biomass, and West et al. (1987) recommend it for the quantitative determination of changes in the fungal biomass in soils. Glucosamine is recommended by Grant and West (1986) and again by West et al. (1987) as an indicator for non-living fungal biomass. Hicks and Newell (1984) and West et al. (1987) found a highly significant relationship between glucosamine content and fungal volume. The glucosamine concentration varied as a function of species and population age between 8.5 and 92.5 $\mu g\ mg^{-1}$ dry weight.

Millar and Cassida (1970) investigated muramic acid concentrations and laboratory grown bacterial populations and reported a value of 3.44 $\mu g\ mg^{-1}$ for Gram-negative and 9.6 $\mu g\ mg^{-1}$ for the Gram-positive populations on a dry weight basis. Moriarty (1975) reported muramic acid concentrations of up to 38 $\mu g\ mg^{-1}$ in bacterial spores. Durska and Kaszubiak (1983) found 0.15–0.39 $mg\ g^{-1}$ in soil, 1.7–7.2 $mg\ g^{-1}$ in humic acid and 4.6–7.4 $mg\ g^{-1}$ in bacterial cultures. All values were given on a dry weight basis. King and White (1977) carried out pulse-labeling experiments and found "that muramic acid is a dynamic biochemical indicator of bacterial biomass in complex microbial assemblages".

The present results indicate that both ergosterol and glucosamine are good indicators of fungal growth. The differences between the amount of ergosterol and of glucosamine was due to the difference in the content of both substances in various species of fungi and to the fact that glucosamine is contained in the exoskeleton of insects and could also be present in the soil.

A significant advantage in the use of glucosamine as an indicator for fungi, as opposed to ergosterol, is that it can be simultaneously extracted and quantified with muramic acid, an indicator for prokaryotic activity. This can be done either with a gas chromatograph (Hicks and Newell 1983) or with high performance liquid chromatography (Zelles 1988).

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