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# Effect of cattle slurry in grassland on microbial biomass and on activities of various enzymes

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**Abstract.** We examined the long-term effects of cattle slurry, applied at high rates, on microbial biomass, respiration, the microbial quotient ($qCO_2$) and various soil enzyme activities. In March, June, July, and October 1991, slurry-amended grassland soils (0–10 cm) contained significantly higher levels of microbial biomass, N mineralization and enzyme activities involved in N, P, and C cycling. With microbial biomass as the relative value, the results revealed that the slurry treatment influenced enzyme production by the microbial biomass. High levels of urease activity were the result not only of a larger microbial biomass, but also of higher levels of enzmye production by this microbial biomass. The ratio of alkaline phosphatase and xylanase to microbial biomass was nearly constant in the different treatments. The metabolic quotient ($qCO_2$) declined with increased levels of slurry application. Therefore it appears that microorganisms in slurry-amended soils require less C and energy if there is no competition for nutrients. The results of this study suggest that urease activity, nitrification, and respiration (metabolic quotient) can be used as indicators of environmental stress, produced by heavy applications of cattle slurry.

**Key words:** Microbial biomass – Soil enzymes – Nitrification – Cattle slurry – Grassland soils

In Austria and other Western countries, intensive arable farming faces several problems related to soil fertility and the environment. One of these urgent problems is the contamination of ground and surface waters, due to intense use of inorganic and organic fertilizers (Laanbroek and Gerards 1991). Cattle slurry has a high N and P fertilizer value and can afford some benefit to plants, but heavy application rates induce N-leaching losses, even from grassland soils (Eder 1991).

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The response by microorganisms to management practices has been measured by estimating the size of the microbial biomass and the activities of various soil enzymes (Stadelmann 1982; Kandeler and Eder 1990; Dick 1992). The ecological significance of these various microbial parameters in soil has been discussed by Nannipieri et al. (1990) and Dick and Tabatabai (1992). But the relationship between the microbial biomass and different soil enzyme activities is not yet fully understood. To describe this relationship Beck (1984) proposed an average activity number called BMK (bodenmikrobiologische Kennzahl). This empiricial index includes intracellular dehydrogenase, catalase, alkaline phosphatase, protease, and amylase activities, and has been significantly correlated with biomass C (Beck 1984). Another biological index of fertility has been proposed by Stefanic et al. (1964; cited in Nannipieri et al. 1990), which includes dehydrogenase activity, catalase activity, and a proportional coefficient. The limitation of these indices is that the enzyme activities appear to react in different ways to the same management practice, pollution agent, and climatic change. Therefore, when these different enzyme activities are summed up in a single index, some of the specific reaction variations are lost. Ecological information on specific aspects of microbial activity may be obtained by calculating the ratio of soil enzyme activity to microbial biomass. An increasing ratio may indicate either increasing enzyme production and enzyme release of microorganisms or an enhanced release of enzymes immobilized on clay or humic colloids in the soil solution. It is clear from laboratory studies that enzymes produced in excess after the addition of organic compounds are rapidly decomposed (Zantua and Bremner 1976, 1977; Nannipieri et al. 1983). In addition, homeostatic mechanisms, which tend to maintain a stable biological composition in the microbial population, affect enzyme activities, as discussed by Nannipieri et al. (1983). According to this hypothesis a long-term increase in enzyme activities, calculated on the basis of the microbial biomass, may reflect enzyme production.

Soil basal respiration reflects the availability of a slow flux of C for microbial maintenance and seems to be a measure of basic turnover rates in soil (Insam et al. 1991). The metabolic quotient ($q$) for $CO_2$ (mg $CO_2$-C mg$^{-1}$ biomass-C h$^{-1}$) may thus be an important parameter in characterizing the effect of different slurry treatments on microbial C.

Our aim was to study the relationship between microbial biomass, respiration, and various other microbial parameters in slurry-amended grassland to ascertain whether one or two microbial parameters were useful indicators of environmental stress. The metabolic quotient (specific respiration), and the enzyme activities, expressed as quotients, were used to describe the product release per unit biomass and unit time.

## Materials and methods

In 1978 an experimental field in Gumpenstein (Austria) was laid out in a block design to study the effect of applications of cattle slurry on plant growth and nutrient cycling in a grassland soil. The soil was classified as an Orthic Luvisol (FAO classification) with 14% clay, 65% silt, 21% sand, 3.56% total C, 0.36 total N, and a pH of 6.6. The mean annual temperature is 6.8 °C and mean annual precipitation is 1013 mm. Soil microbial parameters were investigated four times in 1991 in order to determine the effects of four treatments, (1) control (no fertilization); (2) cattle slurry at 96 N kg$^{-1}$ ha$^{-1}$ year$^{-1}$; (3) cattle slurry at 240 kg N kg$^{-1}$ ha$^{-1}$ year$^{-1}$; and (4) cattle slurry at 480 kg N kg$^{-1}$ ha$^{-1}$ year$^{-1}$. The cattle slurry dressing was split into three equal doses, applied on 10 April 1991, 13 June 1991, and 24 July 1991.

Soil samples (0–10 cm) were taken in March, June, July, and October 1991 from four replicate plots of each treatment. Field-moist soil samples were stored in plastic bags at −20 °C. After the storage period the samples were allowed to thaw at 4 °C for about 3 days, sieved (<2 mm), stored again in plastic bags at 4 °C, and analyzed within 2 weeks. All analytical results were calculated on the basis of the oven-dry (105 °C) weight of soil.

Microbial biomass was determined by the method of Anderson and Domsch (1978). Respiration was measured by titration after trapping $CO_2$ in 0.05 $M$ NaOH and adding $BaCl_2$ (Jäggi 1976). N mineralization under anerobic conditions and potential nitrification were measured according to Keeney (1982) and Berg and Rosswall (1985), respectively. The measurements of various soil enzyme activities were based on the release and quantitative determination of the product in the reaction mixture when soil samples were incubated with their respective substrates and buffers or aqueous solutions. Protease, deaminase, urease, alkaline phosphomonoesterase, xylanase, arylsulfatase, and dehydrogenase activities were determined as described by Schinner et al. (1991). Caseine, arginine, urea, disodium phenylphosphate, xylan, $p$-nitrophenol, and triphenyltetrazoliumchloride were used as substrates for the enzyme assays.

The results of the microbial biomass and soil microbial processes are given as arithmetic means of data from four plots. Analyses of variance were performed to determine the variance attributed to the effect of fer-

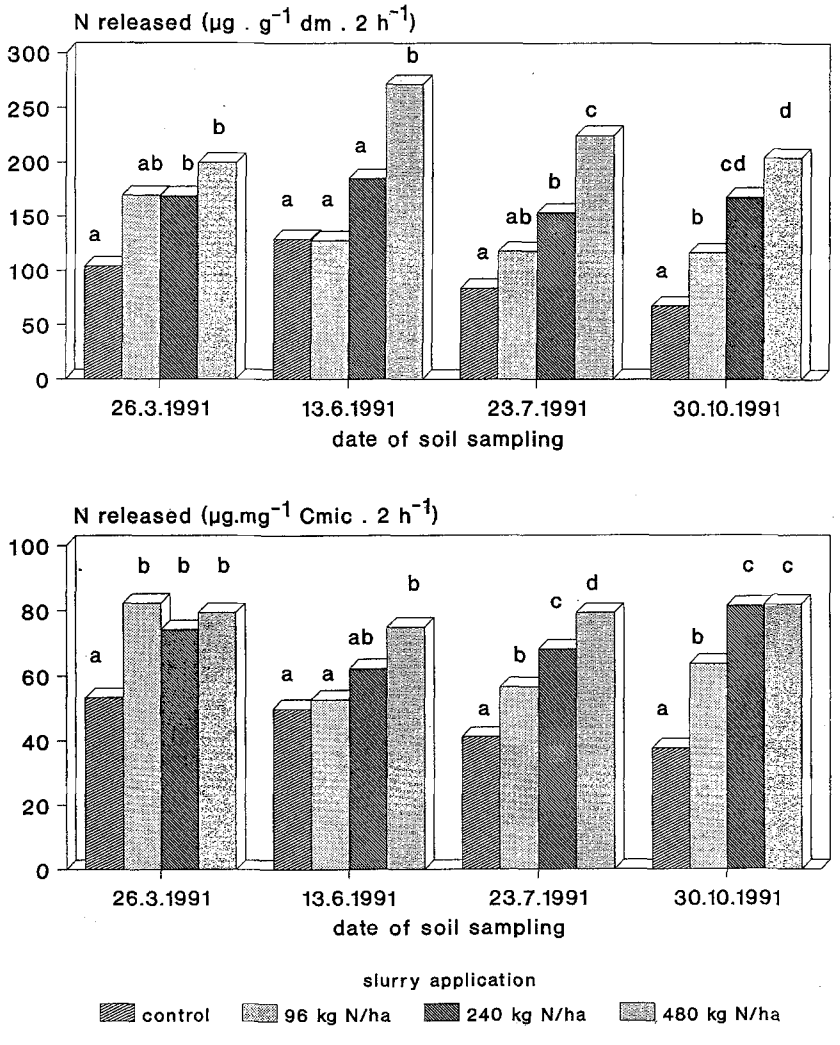


**Fig. 1.** Urease activity (**a**) and the ratio of urease activity to soil microbial biomass (**b**) in grassland soil plots treated with different rates of cattle slurry. *dm*, Dry matter; *Cmic*, microbial biomass C. *Bars* with the same alphabetical are not significantly different at $P<0.05$

tilization with cattle slurry. All calculations were made by using the SPSS package (Version 4, Microsoft Corp., 1988).

## Results and discussion

In March, June, July, and October 1991 the slurry-amended soils (0–10 cm) showed higher potential nitrification rates than the control plots (Table 1). Similar results were obtained with the estimates of microbial biomass, and urease, xylanase and, to some extent, alkaline phosphomonoesterase activities (Figs. 1a–4a). Incorporation of organic materials into soil promotes microbial growth, with a consequent increase in enzyme activities (Zantua and Bremner 1976; Nannipieri et al. 1983). The increase in microbial biomass observed in the slurry-amended soils of the present study was probably due to the input of easily available substrates. Similar results were obtained after manuring or the incorporation of residues into various agricultural soils (Kandeler and Eder 1990; Martens et al. 1992). When the deviation in the microbial parameters of the heavily slurry-amended grassland was calculated as a percentage of the corresponding value for the control plot, urease activity and nitrification potential showed the highest increase after the application of slurry (Fig. 5). Probably the greater supply of $NH_4^+$ in the slurry-amended plots compared with the control soil, due to the ammonification process, was the cause of the increase in nitrification of slurry-

**Table 1.** Seasonal fluctuation of potential nitrification rates in grassland soil plots treated with different rates of cattle slurry

| Date of sampling | Potential nitrification rates (ng N $g^{-1}$ soil dry matter per 5 h) | | | |
|---|---|---|---|---|
| | Control | 96 kg N $ha^{-1}$ | 240 kg N $ha^{-1}$ | 480 kg N $ha^{-1}$ |
| 26 March 1991 | 539.2 ± 133.1 | 648.9 ± 94.4 | 736.5 ± 48.4 | 890.8 ± 76.0 |
| 13 June 1991 | 322.4 ± 102.5 | 326.5 ± 53.8 | 484.8 ± 25.1 | 545.1 ± 47.9 |
| 23 July 1991 | 502.6 ± 167.3 | 626.5 ± 70.8 | 665.5 ± 53.8 | 805.6 ± 41.8 |
| 30 October 1991 | 350.2 ± 124.3 | 466.8 ± 43.1 | 731.8 ± 54.9 | 930.8 ± 51.6 |

Results are expressed as means ± SD, $n = 4$ replicated plots. Effects of treatment and time of sampling are significant at $P < 0.001$

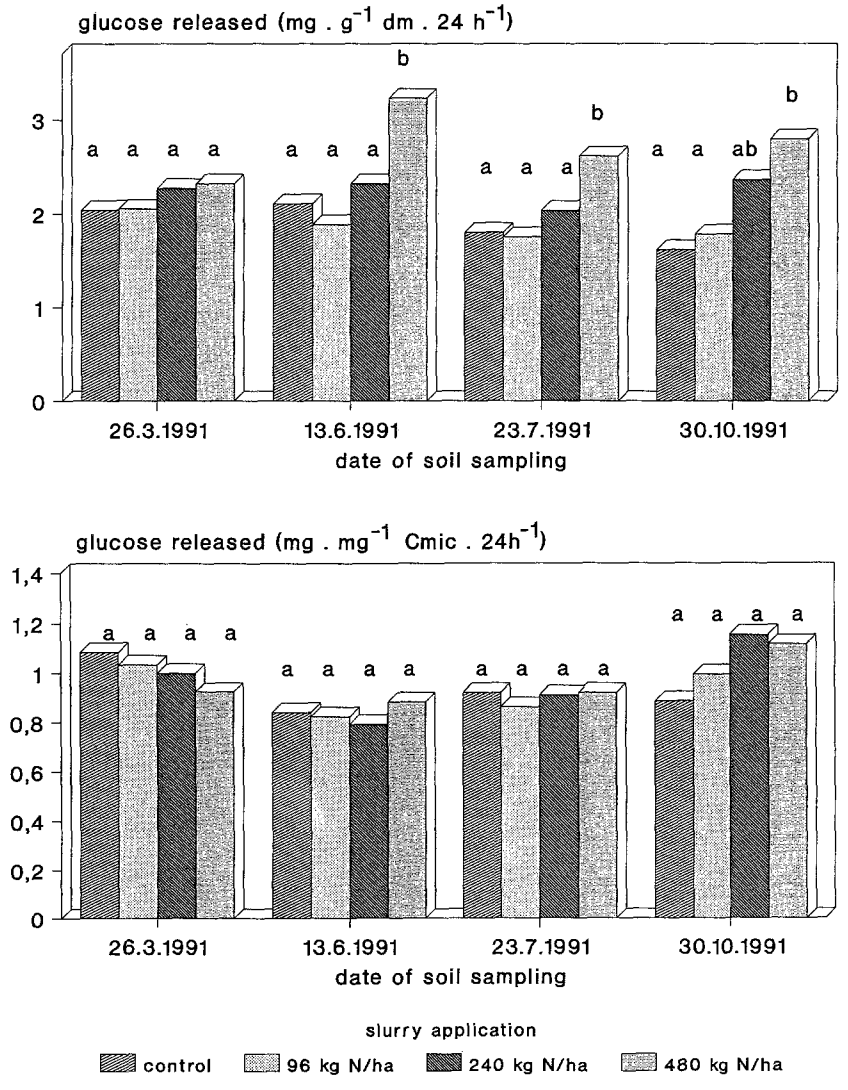


**Fig. 2.** Xylanase activity (**a**) and the ratio of xylanase activity to soil microbial biomass (**b**) in grassland soil plots treated with different rates of cattle slurry. For other explanations, see Fig. 1

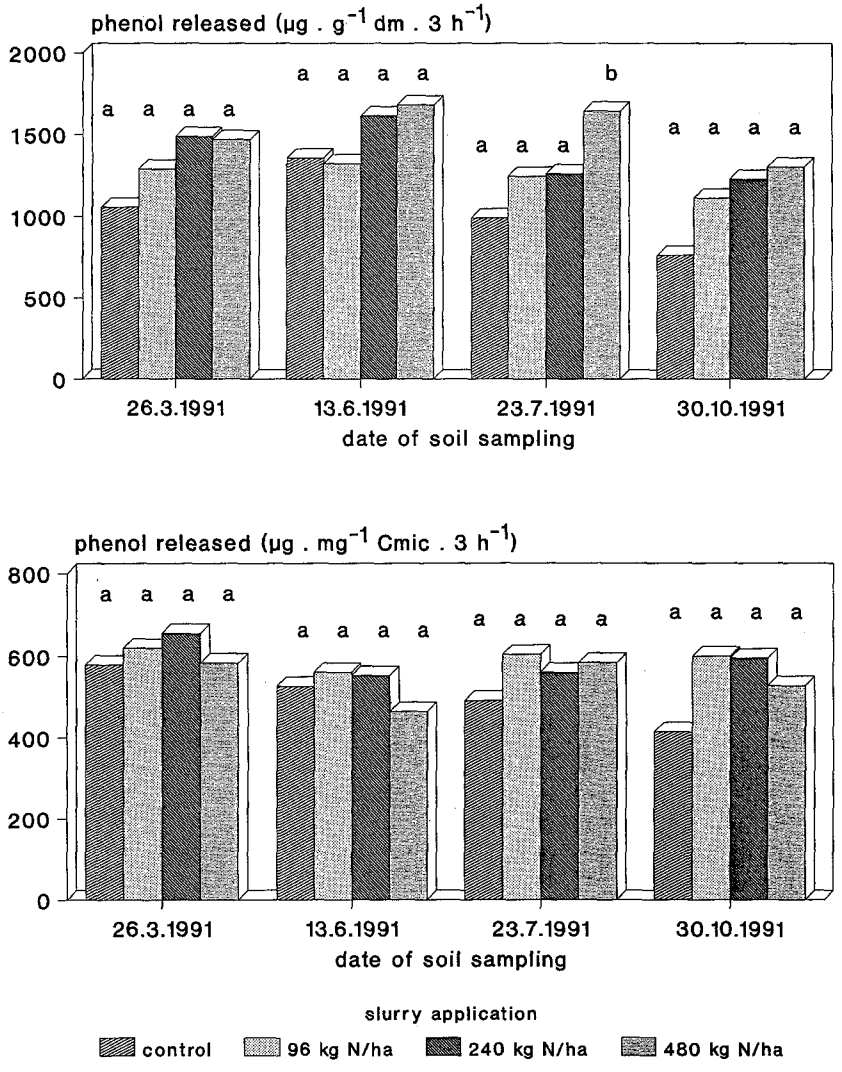


**Fig. 3.** Alkaline phosphomonoesterase activity (**a**) and the ratio of alkaline phosphomonoesterase activity to soil microbial biomass (**b**) in grassland soil plots treated with different rates of cattle slurry. For other explanations, see Fig. 1

amended plots. Estimating urease activity and nitrification in slurry-amended soils seems to be a reasonable approach to determining the environmental stress produced by heavy applications of cattle slurry.

Ratios of enzyme activities to microbial biomass are shown in Figs. 1b–3b. These results indicated that enzyme activities involved in N cycling increased while xylanase and phosphomonoesterase did not change. A larger microbial biomass may produce more urease, which is excreted into soil and is adsorbed onto clay and humic particles. Therefore, the high level of urease activity in slurry-amended grassland is not only the result of a larger microbial biomass, but also of a higher rate of enzyme production by this microbial biomass. In contrast, the size of the microbial biomass and the level of xylanase activity were closely correlated in soils treated with different rates of cattle slurry. As a consequence, the ratio of xylanase to microbial biomass did not change in the slurry-treated plots (Fig. 2b).

Soil respiration was decreased significantly ($P<0.05$) by the slurry treatment (Fig. 5). In addition, the metabolic quotient ($qCO_2$) was significantly lower on slurry-amended grassland soils than on the control plots (Fig. 4b). Similar results were obtained by Insam et al. (1991) in soils treated with inorganic fertilizers. In natural ecosystems the metabolic quotient is related to soil development and decreased with ecological succession (Insam and Domsch 1988; Insam and Haselwandter 1989). Our results from agroecosystems showed that microorganisms in slurry-amended soils require less C and energy when they are not competing for nutrients.

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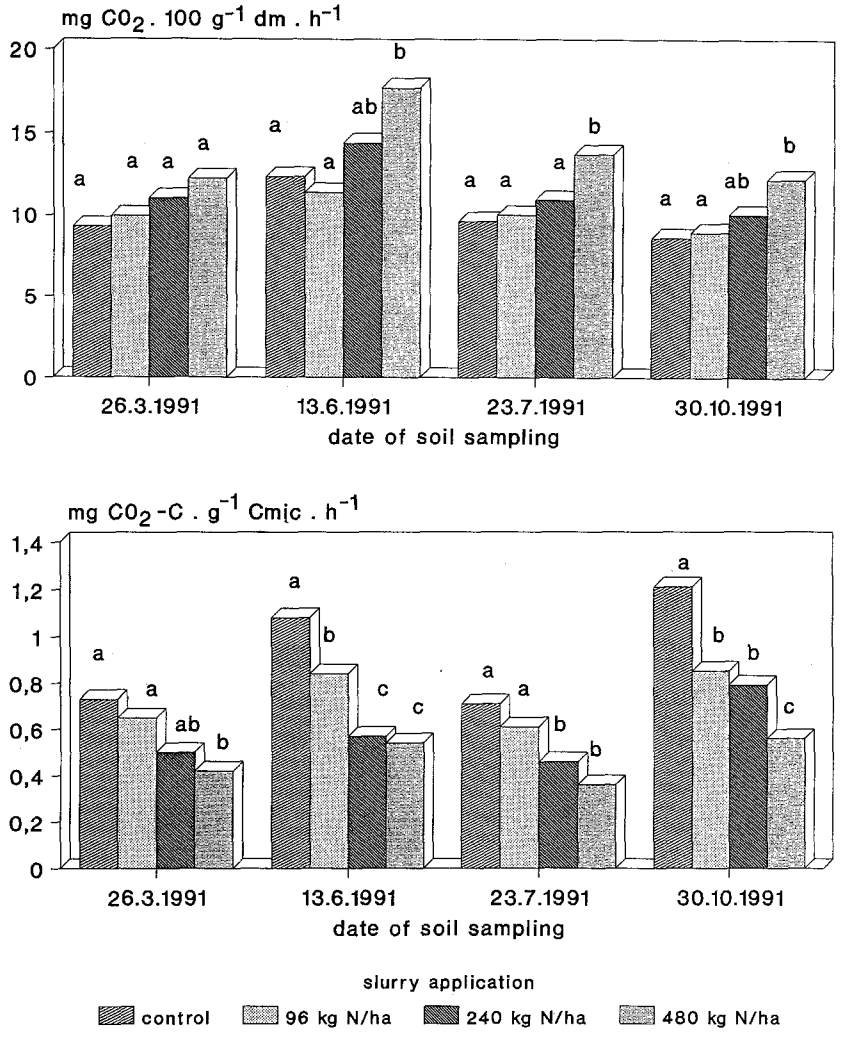


**Fig. 4.** Sesonal fluctuation in substrate-induced respiration (**a**) and specific respiration (metabolic quotient) (**b**) in grassland soil plots treated with different rates of cattle slurry. For other explanations, see Fig. 1

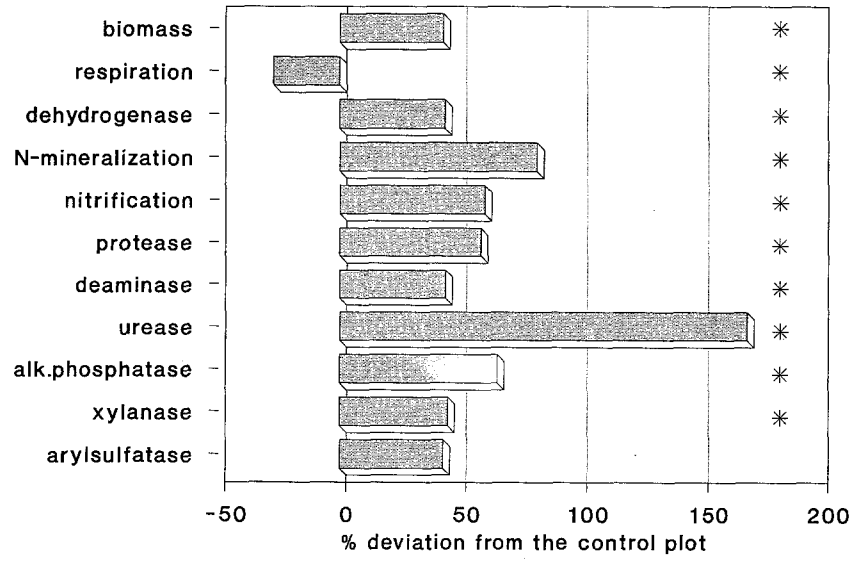


**Fig. 5.** Microbial parameters of the heavily treated soil (480 kg N $ha^{-1}$) expressed as percentages of the control values. *alk.*, Alkaline. $*P<0.05$

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