The influence of organic and mineral nitrogen fertilizers on microbial biomass nitrogen and extractable organic nitrogen in long-term experiments with maize

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ABSTRACT

Microbial biomass nitrogen and extractable organic nitrogen in extractions by 0.05M K_2SO_4 and 0.01M $CaCl_2$ were studied in a long-term experiment with successive growing of silage maize. The highest content of microbial biomass nitrogen was measured for manure treatment, by 38–133% higher than for the control. In treatments with applications of mineral nitrogen fertilizers microbial biomass N was lower on average by 22–30% against the control. Extractable organic nitrogen was also lower in treatments with mineral N fertilizers compared to the control: by 23% in ammonium sulphate treatment and by 29% in DAM. The highest content of extractable organic nitrogen was determined for manure treatment. There was a positive correlation (r = 0.44–0.9) between microbial biomass nitrogen and extractable organic nitrogen in the extractions by 0.01M $CaCl_2$ and 0.05M K_2SO_4.

Keywords: long-term experiment; maize; microbial biomass nitrogen; extractable organic nitrogen

Soil micro-organisms play a key role in nutrient cycling in the soil. Microbial processes are an integral part of the functions of ecosystems connected with nutrient cycling, fertilization and organic matter transformation. Many studies (Schnurer et al. 1985, Anderson and Domsch 1989, Ross and Tate 1993) reported that microbial biomass and microbial activity are closely related with the content of organic matter that is positively influenced by organic matters such as post-harvest residues and organic manure. Microbial biomass is in positive correlation with the amount of organic matters supplied in a longer period, but it also responds to a single application of organic matter (Ocio et al. 1991). If only nitrogen fertilizers are applied, the rate of organic matter mineralization increases, leading to a decrease in the content of easily decomposable organic matter in the soil that is related with a decrease in microbial biomass content (Collins et al. 1992, Lovell et al. 1995).

MATERIAL AND METHODS

Microbial biomass nitrogen and extractable organic nitrogen were investigated in a long-term stationary experiment with silage maize. In 1990 a stationary experiment was established in a field of an experimental station at Červený Újezd; the station is a part of the Agronomic Faculty of Czech University of Agriculture in Prague. Six treatments with different nitrogen fertilization were used in the experiment (Table 1). A detailed description of the experimental method is published in Balík's et al. (2003) communication.

Soil samples were taken from the topsoil profile of 0–30 cm during maize harvest (in September). Samples collected in 1997–2001 were used for the purposes of this study. Soil samples were homogenized after their collection and stored in a freezing box at –18°C until analyzed. Microbial biomass nitrogen and extractable organic nitrogen in the samples were determined in extractions by 0.05M K_2SO_4 and extractable organic nitrogen was determined in extractions by 0.01M $CaCl_2$.

Microbial biomass nitrogen was determined by a fumigation-extraction method (FE-N) (Brookes et al. 1985). The analysis of each sample was repeated twice. The weighed-out amount of thoroughly homogenized moist soil was 30 g. First of all, pre-extraction with 100 ml of 0.05M K_2SO_4 was done in all samples (Widmer et al. 1989, Mueller et al. 1992, Joergensen et al. 1994). After 15-minute shaking of samples the supernatant was centrifuged. The content of mineral nitrogen and total extractable nitrogen was measured in the extraction. Extractable organic nitrogen was calculated as a difference in the values of total extractable nitrogen and mineral nitrogen.

To determine microbial biomass N, immediately after pre-extraction a half of the samples was extracted with 100 ml of 0.5M K_2SO_4. 45-minute shaking of samples was followed by centrifugation. The content of total extractable nitrogen was determined in the extraction. The other half of the samples was chloroform fumigated. Samples were put into an exsiccator where a dish with 30 ml chloroform $CHCl_3$ was placed. A vacuum pump was used to induce vacuum until chloroform started boiling. Fumigation took place in darkness, at 25°C for 24 hours. Similar-

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Table 1. Experiment layout in Červený Újezd locality

Treatment No.	Treatment	Treatment designation	Σ kg N/ha per year in mineral fertilizers	Σ kg N/ha for 1990/2001	Time of application (month)
1	control	control	–	–	–
2	ammonium sulphate	AS	120	1320	XI
3	DAM	DAM	120	1320	IV
4	DAM + winter wheat straw	DAM + straw	120	1481	straw X DAM IV
5	farmyard manure	manure	–	1975	X
6	black fallow*	fallow	–	–	

*no fertilizers were applied in treatment 6

ly like chloroform-untreated samples, fumigated samples were extracted with 100 ml of 0.5M K_2SO_4. The content of total extractable nitrogen was measured in the extraction after centrifugation. Microbial biomass N was calculated as a difference in N content in fumigated and nonfumigated sample (E_N) using coefficient k_{EN} (microbial biomass N = E_N:k_{EN}). The coefficient k_{EN} indicates an extractable portion of microbial biomass after fumigation. The value k_{EN} = 0.54 was used to calculate microbial biomass N (Brookes et al. 1985, Jenkinson 1988, Joergensen and Mueller 1996).

Extractable organic nitrogen ($CaCl_2$) was calculated as a difference between total extractable nitrogen and mineral nitrogen that were determined in extractions by 0.01M $CaCl_2$ (Houba et al. 1986). 10 g of soil was extracted in 100 ml of the extractant.

Segment flow analysis with colorimetric determination in a SKALAR plus SYSTEM apparatus was used to determine extractable nitrogen.

RESULTS AND DISCUSSION

Microbial biomass nitrogen ranged from 4.6 to 18.4 mg N/kg. As shown in Figure 1, the year of sampling markedly influenced the content of microbial biomass nitrogen in topsoil. The highest values measured in 1997 are almost twice as high as microbial biomass nitrogen determined in 2000 and 2001. Šantrůčková (1993) reported that yearly variations in microbial biomass are a result of the complex of physical, chemical, biological and anthropogenic factors, and a result of variability in these factors, which along with a short generation time of micro-organisms is reflected in the large fluctuations of microbial biomass content. Biomass variability is influenced by the conditions of the year concerned while the fluctuations are marked under high variability of environmental factors, e.g. fast and marked alternations of moisture, temperature, effect of NO_3^- ions, etc. The results document that the contents of microbial biomass nitrogen in the particular years corresponded with each other; statistical evaluation demonstrated close correlations of the values measured in the particular years. Correlation coefficients r = 0.65–0.91 indicated statistical significance in all cases (p = 0.05).

As shown by the evaluation of the particular treatments, the highest content of microbial biomass nitrogen was determined for manure application. Microbial biomass N was higher by 38 to 133% in the years of observation compared to the control. In the treatments with mineral nitrogen fertilizers microbial biomass nitrogen was lower than in the control. The application of ammonium sulphate resulted in microbial biomass N lower by 30% compared to the control and for DAM treatment it

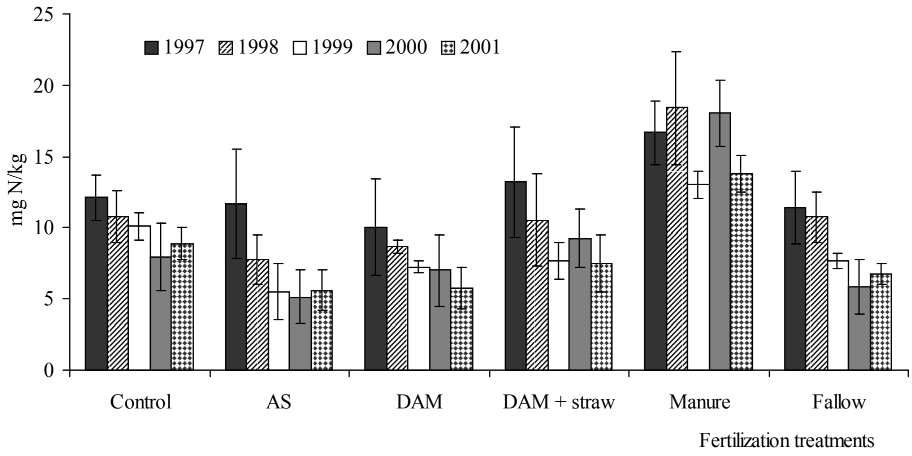

Figure 1. Content of microbial biomass nitrogen (mg N/kg)

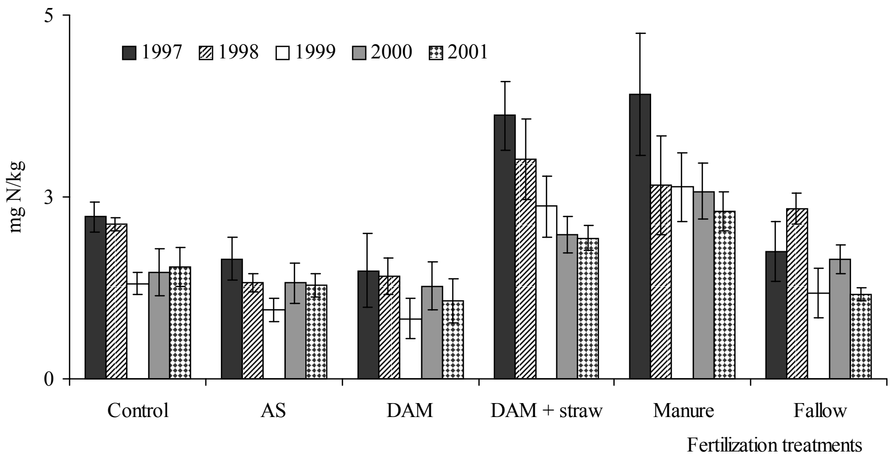

Figure 2. Content of extractable organic nitrogen in 0.05M K_2SO_4 (mg N/kg)

was 78% of microbial biomass N in the control. Lovell and Jarvis (1998) reported that especially regular and long-term applications of nitrogen led to a decrease in the content of soil microbial biomass. But Coote and Ramsey (1983) stated that nitrogen fertilizers had an indirect positive influence on soil microbial biomass by the increased crop yield, and subsequently by a higher return of organic N and C into the soil through post-harvest residues. However, the results of our experiments indicate that in the field with silage maize a relatively low amount of organic residues returns into the soil in form of post-harvest residues. These are roots and low stubble in our experiment. It is to note that maize is a crop with negative balance of organic matters (Körschens and Schulz 1999).

Microbial biomass nitrogen was on the level of the control for DAM + straw treatment. On average by 19% higher content of microbial biomass nitrogen was determined for DAM + straw treatment compared to DAM application. This finding corresponds to conclusions drawn by Ocio et al. (1991), who measured by 18% higher content of microbial biomass N (FE-N) against the control in experiments with straw ploughing-in, a year after its application.

Microbial biomass nitrogen in fallow land markedly corresponds to the content of biomass N in the control in the years of observation. But microbial biomass N in the control in Červený Újezd locality was found to be higher by 17% on average compared to fallow land.

The measured values of extractable organic nitrogen in the extraction by 0.05M K_2SO_4 were relatively low and ranged from 0.8 to 3.9 mg N/kg (Figure 2). Extractable organic nitrogen for applications of mineral nitrogen fertilizers was lower in comparison with the control. For AS and DAM treatments extractable organic nitrogen was on the level of 77 and 71%, respectively, of extractable organic N in the control (1.73 mg N/kg). In all years of observation the differences in microbial biomass nitrogen between the control and fertilization treatments were statistically significant. Manure treatment had the highest content of extractable organic nitrogen. It was higher on average by 66% compared to the control. The application of straw in DAM + straw treatment also contributed to an increase in extractable organic nitrogen by 50% compared to the control. In comparison with DAM treatment extractable organic nitrogen was higher by 80%. There were no statistically significant differences in extractable organic nitrogen between fallow land and control although extractable organic nitrogen was lower on average by 9%.

Extractable organic nitrogen in the extraction by 0.01M $CaCl_2$ ranged from 1.4 to 5.8 mg N/kg (Figure 3).

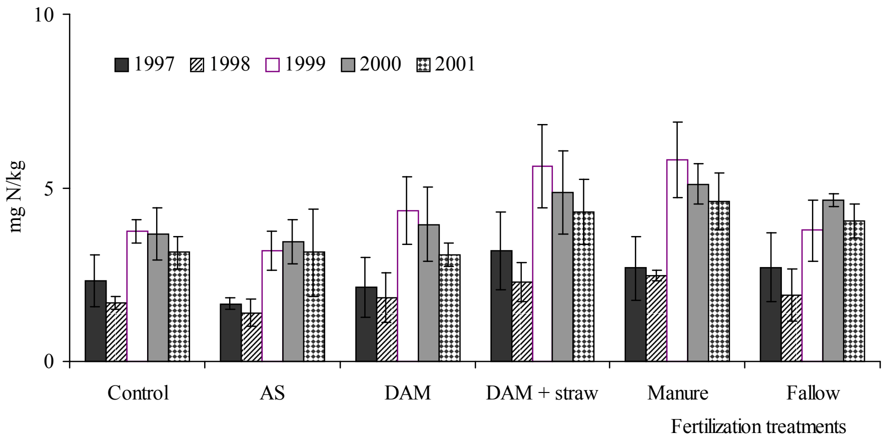

Figure 3. Content of extractable organic nitrogen in 0.01M $CaCl_2$ (mg N/kg)

Table 2. Relations between microbial biomass N and extractable organic nitrogen (correlation coefficients)

Year	Compared values		
	A	B	C
1997	0.44	0.90	0.23
1998	0.79	0.77	0.86
1999	0.55	0.55	0.79
2000	0.68	0.84	0.82
2001	0.63	0.87	0.91

A = microbial biomass N and extractable organic N in extraction by 0.01M $CaCl_2$
B = microbial biomass N and extractable organic N in extraction by 0.05M K_2SO_4
C = extractable organic N in extraction by 0.01M $CaCl_2$ and extractable organic N in extraction by 0.05M K_2SO_4

In AS treatment extractable organic nitrogen was 90% of extractable organic N in the control. In DAM treatment the average content of extractable organic nitrogen was comparable with the control. Different values were measured in the years of observation. In 1997 and 2001 extractable organic N was lower in DAM treatment, but it was higher in the other years. The differences were not statistically significant. For manure treatment, extractable organic nitrogen was higher on average by 40% than in the control. The application of straw in DAM + straw treatment increased extractable organic nitrogen by 38% compared to the control. Extractable organic nitrogen in fallow land was higher on average by 17% than in the control, but the differences in the values were not statistically significant.

Based on the statistical evaluation of measured values a positive correlation was determined between microbial biomass nitrogen and extractable organic nitrogen in extractions by 0.01M $CaCl_2$ and 0.05M K_2SO_4. Regression analysis on a 95% reliability level ($\alpha = 0.05$) was used to evaluate the relations between these parameters. Correlation coefficient r was used to determine the closeness of the relations.

Table 2 shows a closer relation between microbial biomass nitrogen and extractable organic nitrogen and 0.05M K_2SO_4 ($r = 0.55$–0.9), compared to the extraction of organic nitrogen by 0.01M $CaCl_2$ ($r = 0.44$–0.79). There was also a close correlation ($r = 0.86$–0.91) between extractable nitrogen in 0.01M $CaCl_2$ and extractable organic nitrogen and 0.05M K_2SO_4, except for 1997, when the correlation coefficient was $r = 0.23$.

As reported by Ocio et al. (1991), microbial biomass is in positive correlation with the amount of organic matters supplied in a longer period, and it also responds to a single supply of organic matter. Hence it is obvious that microbial biomass depends on the content of easily decomposable organic matters to a larger extent than on the content of total organic C and N. Soil microbial biomass responds to a change in the supply of organic matters into the soil more readily than soil organic matter (Powlson and Jenkinson 1981).

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ABSTRAKT

Vliv organických a minerálních dusíkatých hnojiv na obsah dusíku mikrobiální biomasy a extrahovatelného organického dusíku v dlouhodobých pokusech s kukuřicí

V dlouhodobém pokusu s opakovaným pěstováním silážní kukuřice byl sledován obsah dusíku mikrobiální biomasy a obsah extrahovatelného organického dusíku ve výluhu 0,05M K_2SO_4 a 0,01M $CaCl_2$. Nejvyšší obsah dusíku mikrobiální biomasy byl stanoven na variantě hnojené hnojem, v porovnání s kontrolou byl o 38–133 % vyšší. Na variantách hnojených pouze minerálními dusíkatými hnojivy byl obsah N mikrobiální biomasy v průměru o 22–30 % nižší oproti kontrole. Také obsahy extrahovatelného organického dusíku byly nižší na variantách s minerálními N hnojivy ve srovnání s kontrolou, na variantě se síranem amonným o 23 % a s DAM o 29 %. Nejvyšší obsah extrahovatelného organického dusíku byl stanoven na variantě hnojené hnojem. Mezi obsahem dusíku mikrobiální biomasy a obsahem extrahovatelného organického dusíku ve výluhu 0,01M $CaCl_2$ a 0,05M K_2SO_4 byla stanovena kladná korelace (r = 0,44–0,9)

Klíčová slova: dlouhodobý pokus; kukuřice; dusík mikrobiální biomasy; extrahovatelný organický dusík

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