The influence of fertilisation and crop rotation on soil microbial characteristics in the long-term field experiment

O. Mikanová, M. Friedlová, T. Šimon

Crop Research Institute, Prague-Ruzyně, Czech Republic

ABSTRACT

Soils were sampled from the plots with four variants of fertilisation: Nil – without fertilisation, NPK – mineral fertilisation, FYM – farmyard manure, FYM + NPK – farmyard manure with mineral fertilisation, and two variants of crop rotation: field IV – classical 9-year crop rotation, field B – 2-year rotation of alternative growing. Determination of urease, CFU of *Azotobacter* spp. and potential nitrogenase activity was conducted during the period 1999–2004. The urease activity was positively affected by manure fertilisation (FYM) and by the combination of FYM + NPK. The statistically significantly highest counts of *Azotobacter* spp. and the highest nitrogenase activity were determined on field B in variants FYM and FYM + NPK. The results show that there was a higher amount of accessible nitrogen present on field IV than on field B. This might explain the lower counts of *Azotobacter* spp. and therefore the lower nitrogenase activity. According to our results, activity of urease, CFU of *Azotobacter* spp. and potential nitrogenase activity are very closely connected with N inputs.

Keywords: urease; *Azotobacter* spp.; fertilisation; potential nitrogenase activity

Sustainable soil management systems require not only a right choice of cropping methods and ensured supply of nutrients, but also a subsequent soil quality evaluation. It is well known that soil fertility is connected to the biological activity of soil.

Traditional mineral fertilisation reliably increases nutrient content in soil, which increases yield and at the same time positively affects soil fertility. Organic fertilisation, especially manure, compost and biofertilisers have been used in agriculture for a long time. The goal is to increase soil fertility by increasing soil organic matter and active soil microflora content and thus to contribute to the improvement of the soil biological properties.

Long-term soil management requires also monitoring of the biological activity of soil. Soil quality evaluation and the forecast of possible changes undoubtedly belong among the significant issues. Many different parameters documenting the condition of soil microflora and ecologically important processes can be used for evaluating the biological activity of soil (Kubát et al. 1996, Mikanová et al. 1996).

The process of fixing atmospheric nitrogen plays an important role in the nitrogen cycle. Biological fixers of nitrogen support the growth potential of plants, preserve more organic soil nitrogen and other nutrients in the soil – plant system and thus lower fertilisation costs, reduce greenhouse gas emissions and reduce the nitrate runoff (Kennedy et al. 2004).

Enzymatic activity is also considered to be a good indicator of soil quality because it controls a release of nutrients for plants and the growth of microorganisms. Enzymes have various origins and are located in various soil components. Determining specific enzymatic activity can also encompass certain processes occurring in the soil.

The nitrogen cycle process is well characterised by the urease activity (Nayak et al. 2007).

The aim of this paper is to determine how a long-term organic and inorganic fertilisation affects the counts of *Azotobacter* spp., potential nitrogenase activity and urease activity in soil.

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MATERIAL AND METHODS

Soil samples were taken from a long-term field experiment in Prague-Ruzyně, established in 1955. The long-term experiment includes two fields differing in crop rotation and a combination of mineral and organic fertilisation. It focuses on the effect of cropping, organic and mineral fertilisation on the microbiological characteristics that are closely related to nitrogen cycle in soil.

The long-term field experiments are located in Prague-Ruzyně. The altitude of the site is about 352 m above sea level, average annual temperature is 8.1°C and average annual precipitation 450 mm. Soil type is Orthic Luvisol, clay-loam, developed on diluvial sediments mixed with loess.

Four fertilisation treatments were selected: Nil – without fertiliser, NPK – mineral fertilisation, FYM – farmyard manure, and FYM + NPK – farmyard manure with mineral fertilisation. Similar treatments were selected in two fields differing in crop rotation: field IV – classical 9-year rotation (45% cereals, 33% root crops, 22% fodder crops) and field B – alternative growing of spring wheat and sugar beet (50% cereals, 50% root crops) (Kubát et al. 2001). Table 1 shows average doses of nitrogen fertilisers in kg of N per year in individual variants.

Topsoil samples (0–20 cm) were collected in spring and in autumn over the period of 1999–2004. The samples were sieved through 2-mm sieve and stored in refrigerator at 4°C.

Activity of urease was determined according to Kandeler and Gerber (1988). The determination is based on the reaction of sodium salicylate with NH_3 in the presence of sodium dichloroisocyanurate which forms a green-coloured complex under alkaline pH conditions. Sodium nitroprusside is used as a catalyst.

Table 1. Average N doses in kg per year

	Crop rotation	Variants	Average N doses (kg N/ha/years)
Field B	2-year	Nil	0
		NPK	100
		FYM	57
		FYM + NPK	157
Field IV	9-year	Nil	0
		NPK	64.6
		FYM	38.6
		FYM + NPK	103.2

Nil – without fertilisation; NPK – mineral fertilisation; FYM – farmyard manure; FYM + NPK – farmyard manure with mineral fertilisation

Non-symbiotic nitrogen fixation (potential nitrogenase activity) was determined according to Šimek (1993). Fresh soil samples were sieved through 2-mm sieve and weighed into 100 ml incubation flasks – equivalent to 15 g of dry soil. 2 ml of 7.5% glucose solution was added as an energy source for microorganisms. 10% of the volume in flasks was supplemented with the same volume of acetylene. The flasks were incubated at room temperature for 48 h. After the incubation, gas samples were taken into the syringes and the amount of ethylene was analyzed on gas chromatograph.

The number of *Azotobacter* spp. was determined on Ashby agar by standard dilution method.

$N\text{-}NH_4^+$ and $N\text{-}NO_3^-$ were determined in soil extract by 1% K_2SO_4 on a Skalar analyser.

Organic carbon and total nitrogen was determined in air-dried soil samples with Leco CNS 2000 Analyser.

The results were analyzed by ANOVA. Statistical analyses were carried out with the program StatSoft Statistica Cz (Tukey HSD). The columns designed with the same letter do not differ in a statistically significant way ($P = 0.05$).

Analyses were performed (in spring and in autumn over the period of 1999–2004) in three replicates and average values are presented.

RESULTS AND DISCUSSION

The results of urease activity are shown in Figure 1.

The urease activity was positively affected by manure fertilisation (FYM) and the combination of FYM + NPK. Also Kandeler et al. (1999) and Nayak et al. (2007) found that, compared to NPK mineral fertilisation, manure fertilisation increases urease activity. Other authors (Gianfreda and Bollag 1996, Balakrishnan et al. 2007) also showed a stimulating effect of organic fertilisation on the microbial biomass and soil enzyme activities in comparison with inorganic fertilisation or unmanured soil.

The counts of *Azotobacter* spp. colony-forming units (CFU) on Ashby agar are shown in Figure 2.

Potential nitrogenase activity is shown in Figure 3.

The statistically significantly highest counts of *Azotobacter* spp. and the highest nitrogenase

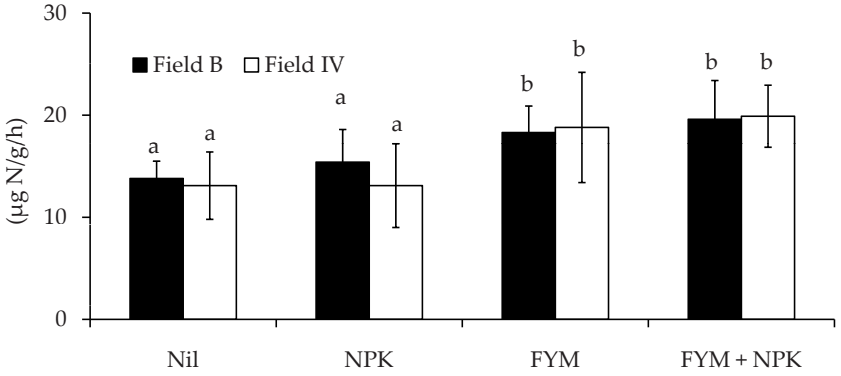

Figure 1. Average values of urease activity in soil samples of the selected variants of the long-term field experiment in Prague-Ruzyně over the period 1999–2004 (µg N/g/h). Values within a column designed with different letters are significantly different at the 0.05 probability level

activity were determined on field B in variants FYM and FYM + NPK.

Counts of *Azotobacter* spp. and potential nitrogenase activity are very closely related to the nitrogen content in soil. According to our results, nitrogen fertilisation in organic form (FYM) increased the counts of *Azotobacter* spp. and subsequently also the potential nitrogenase activity. Inorganic fertilisation (NPK) had no effect on the measured characteristics. Our results are in accordance with those published by Czakó et al. (2007); they found that the addition of 400 metric tons of compost per hectare applied during recultivation significantly increased the counts of *Azotobacter* spp. in dump substrate. Also Kubát et al. (1997) documented increasing counts of free-living nitrogen-fixing bacteria in a long-term experiment in Ruzyně on field B in the FYM + NPK variant as compared to the Nil variant.

The C/N ratio is another important factor for growth and development of *Azotobacter* spp.; it is represented in Figure 4.

Fertilisation increases not only the quantity of nitrogen but, under favourable conditions, also the quantity of carbon. Therefore, despite the fact that the combined organic and mineral fertilisation variant (FYM + NPK) delivered a large amount of nitrogen, the C/N ratio remained balanced.

Furthermore, we determined the amount of accessible nitrogen in form of both nitrate nitrogen, and ammonia nitrogen content. The results are shown in Figures 5 and 6.

The results show that there was a higher amount of accessible nitrogen present on field IV (both ammonia nitrogen and nitrate nitrogen content) than on field B. This would explain the lower counts of *Azotobacter* spp. and therefore the lower nitrogenase activity. Our results are in accordance

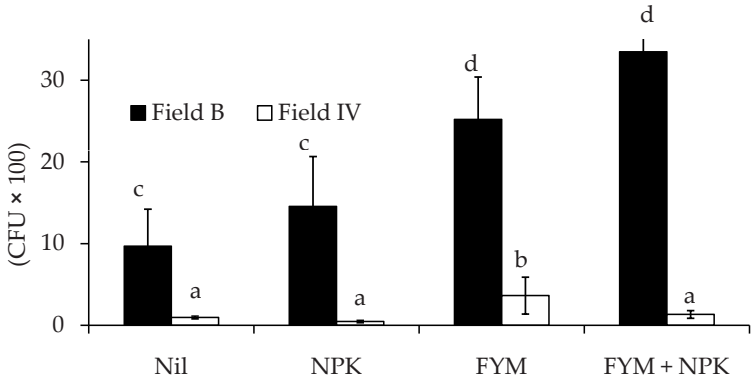

Figure 2. Average number of *Azotobacter* spp. in soil samples of the selected variants of the long-term field experiment in Prague-Ruzyně determined on Ashby agar over the period 1999–2004 (CFU × 10^{-1} g^{-1}). Values within a column designed with different letters are significantly different at the 0.05 probability level

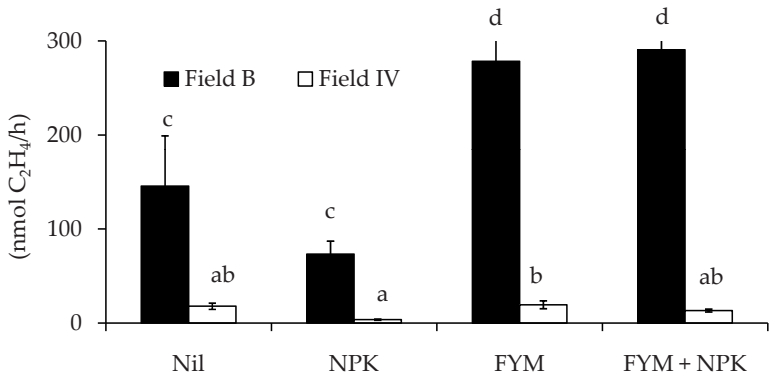

Figure 3. Average values of potential nitrogenase activity in soil samples from the selected variants of the long-term field experiment in Prague-Ruzyně over the period 1999–2004 (nmol C_2H_4/48 h). Values within a column designed with different letters are significantly different at the 0.05 probability level

with those of other authors who claim a negative effect of accessible nitrogen on average counts of the free-living N_2-fixing bacteria. For example, Kubát et al. (1999) reported an elimination of the free-living diazotrophic bacteria (*Azotobacter* spp.) in mineral fertilisation variants in a long-term bare fallow experiment. Similarly, Patra et al. (2007) document a negative correlation between $N\text{-}NO_3^-$ production and N_2-fixation. Buresh et al. (1980) and Yoch and Whiting (1986) mention that among many environmental variables, oxygen and mineral nutrients are considered to be the most important for N_2-fixation. Of the nutrients, $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$ may regulate this process significantly. Also Narula et al. (2007) document that maximum dry matter of cultivated plants was obtained in *Azotobacter chroococcum* inoculation without N treatments compared with a full dose of 90 kg/ha nitrogen as NH_4NO_3.

Inorganic and organic fertilisation, primarily with nitrogen, not only increases soil fertility but also influences its chemical, physical and biological properties. These changes can be seen well in long-term field experiments. There is no doubt that mineral fertilisation increases nutrient content in the soil, thus influencing soil quality in terms of soil productivity (fertility). Organic fertilisation, using particularly manure and compost, improves soil fertility by increasing the content of soil organic matter and the activity of soil microflora, and helps enhance the biological processes of soil. Ondrášek and Čunderlík (2008) concluded that the effects of fertilisers, especially manure application, on microbial parameters of soil were positive. The manure application enriches the soil not only with many organic substances but also with numerous forms of microorganisms enhancing and intensifying the biological activity of soil.

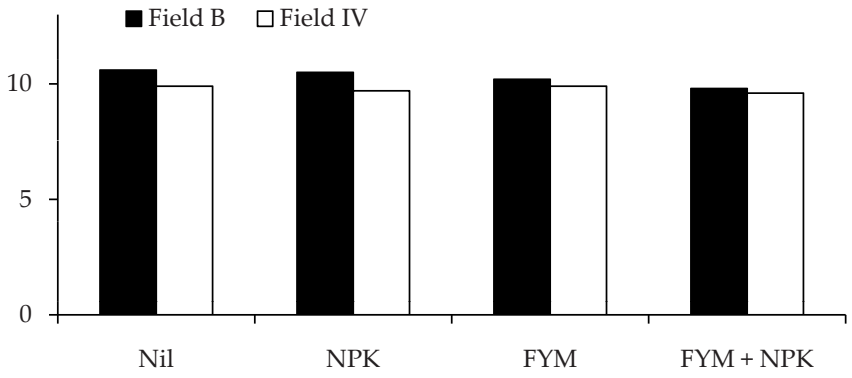

Figure 4. Average values of the ratio of organic carbon to total nitrogen content in soil samples from the selected variants of the long-term field experiment in Prague-Ruzyně over the period 1999–2004

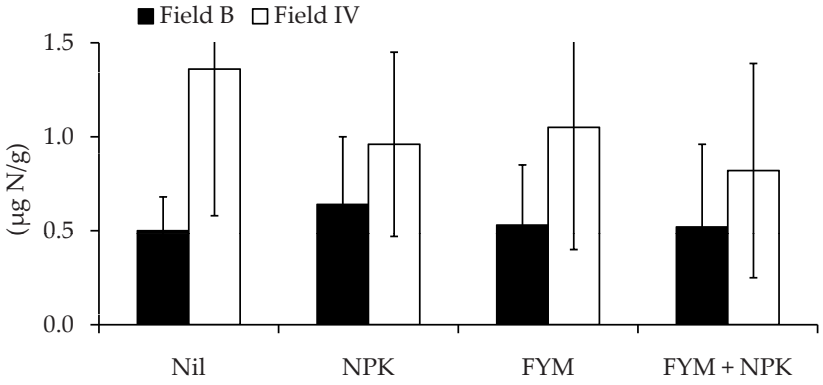

Figure 5. Average values of ammonia nitrogen content (N-NH_4) in soil samples from the selected variants of the long-term field experiment in Prague-Ruzyně over the period 1999–2004 (µg N/g)

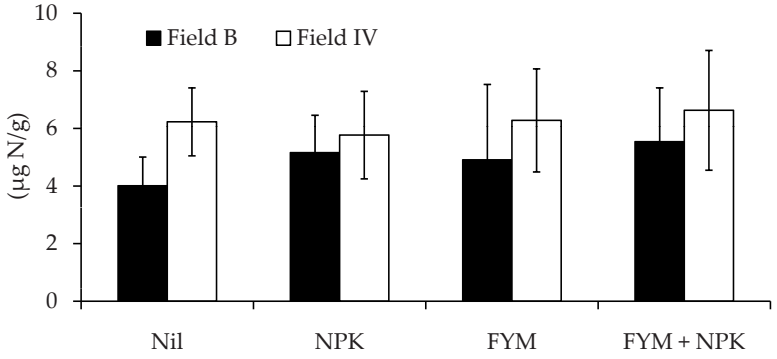

Figure 6. Average values of nitrate nitrogen (N-NO_3) content in soil samples from the selected variants of the long-term field experiment in Prague-Ruzyně over the period 1999–2004 (µg N/g)

According to our results, urease activity was positively influenced by manure fertilisation (FYM + 0) and combined FYM + NPK fertilisation on both field B and field IV. The results show that urease activity well characterises the nitrogen cycle processes.

The process of fixing atmospheric nitrogen plays an important role in the nitrogen cycle; atmospheric nitrogen-fixing bacteria support plant growth by supplying nitrogen and reducing fertilisation costs.

The counts of *Azotobacter* spp. and potential nitrogenase activity are closely related to each other as well as to nitrogen content in soil. According to our results, nitrogen fertilisation in the organic form (manure) increased the counts of *Azotobacter* spp. and subsequently also the potential nitrogenase activity. It is obvious that the suitable conditions for *Azotobacter* spp. development in soil, especially a sufficient supply of organic matter, increases the supply of nitrogen to soil. It is, however, necessary to monitor the content of accessible nitrogen in soil in the form of both ammonia nitrogen and nitrate nitrogen. According to our results, the statistically significantly higher content of mineral nitrogen on field IV had an inhibitory effect on the counts of *Azotobacter* spp., thereby negatively influencing potential nitrogenase activity.

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Corresponding author:

Ing. Olga Mikanová, Ph.D., Výzkumný ústav rostlinné výroby, v. v. i., Drnovská 507, 161 06 Praha 6-Ruzyně, Česká republika
phone: + 420 233 022 273, e-mail: mikanova@vurv.cz