Studies of the influences of different N fertilizers and Microbion UNC bacterial fertilizer on the nutrient content of soil

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Keywords: nitrogen fertilizers, bacterial fertilizer, soil, nutrients

SUMMARY

A field experiment was conducted to examine the effects of different nitrogen fertilizers in combination with bacterial fertilizer on nutrient uptake of horseradish and plant available nutrients of the soil. Three different N fertilizers, ammonium-nitrate, urea and calcium-nitrate (116 kg ha^{-1} N) in combination with Microbion UNC bacterial fertilizer (2 kg ha^{-1}) were applied as treatments in a randomized complete block design in three replications. In this paper we presented the results of soil measurements. The soil of the experimental area was chernozem with medium sufficiency level of N and P and poor level of K.

Our main results:

The amount of 0.01M $CaCl_2$ soluble inorganic nitrogen fractions, NO_3^--N and NH_4^+-N and also the quantity of soluble organic-N were almost the same in the soil. N fertilizers significantly increased all the soluble N fractions. The amount of NO_3^--N increased to the greatest extent and the increase of organic N was the slightest. We measured the largest $CaCl_2$ soluble NO_3^- -N and total-N contents in the plots treated with ammonium-nitrate, the largest NH_4^+-N in the plots treated with calcium-nitrate and the largest organic-N fraction in plots treated with urea.

Bacterial inoculation also increased both soluble inorganic nitrogen forms and also total-N content of soil compared to the control. In the case of combined (artificial and bacterial fertilizer) treatments we measured lower NO_3^--N, organic-N and total-N compared to the values of plots having only nitrogen fertilizer treatments. On the contrary in the plots with combined treatments the $CaCl_2$ soluble NH_4^+-N content of soil in more cases were higher than that of values with artificial fertilizer treatment.

As a function of calcium-nitrate application increased AL-P_2O_5 and AL-K_2O values were measured compared to control. Microbion UNC supplement of calcium nitrate yielded also increase in AL-P_2O_5 and AL-K_2O values, till then supplement of ammonium-nitrate fertilizer yielded a decrease in these values compared to the control.

All nitrogen fertilizers resulted in a significant decrease in AL-Mg content of soil compared to the control. Nevertheless bacterial fertilizer increased AL-Mg values in any cases.

INTRODUCTION

The use of the nitrogen fertilizers is a standard practice in the plant production system. Nitrogen fertilizers may contain nitrogen in different chemical forms, for example, nitrate, ammonium. It has long been observed that ammonium and nitrate differ in their effects on the soil and on the growth and chemical composition of plants (Lewis and Chadwick, 1983; Maier et al., 2002). Plants take up nitrogen as nitrate (NO_3^-) and ammonium (NH_4^+) ions. Moreover, NO_3^- and NH_4^+ induce a net release of OH^- and H^+ ions, respectively (Haynes, 1990; Hinsinger et al., 2003). Hence they may change the rhizosphere pH in different way and pose the distinct influence on nutrient availability in soil (Jalloh et al., 2009). When urea is applied as nitrogen fertilizer, it causes little pH change (Nye, 1981; Hayness, 1990), but it can be hydrolyzed by microbially, produced NH_4^+ and NO_3^-, also. Application of different N fertilizers may change the rhizosphere pH in different manner, so may effects differently on the life of soil and on the solubility and availability of nutrients.

Nowadays the application of nitrogen fertilizers becomes more and more one-sided. Not balanced use of chemical fertilizers may cause environmental pollution and ecological damage (Ghost and Bhat, 1998; Kádár et al., 2007). Increasing concern over nitrate contamination of soils, nitrate leaching in groundwater may require prudent and rational N application in crop production, while maintaining optimal productivity (Gutezeit and Fink, 1999).

For reducing chemical fertilizers application an alternative method may be developed. For this reason, environmental friendly products such as bacterial fertilizers should be used. The bacterial fertilizers are products containing different types of microorganisms (Hegde et al., 1999; Vessey, 2003, Vance, 1997), for example nitrogen fixing, phosphate solublizing bacteria, cellulolytic microorganisms. They may promote plant growth and health by various means such as mineralization of nutritional elements, nodulation and nitrogen fixation (Malboobi, 2009) and they may augment the availability of nutrients to the plants. Nevertheless the performance of biofertilizers is severely influenced by both biotic and abiotic environmental conditions also.

In Hungary several studies were performed on the effect of N fertilizer applications, but few ones have been dealing with the bacterial fertilizer application and especially on the combined application of nitrogen and bacterial fertilizers and their effects on the nutrient content and the nutrient availability of different soils (Kincses et al., 2008).

The aim of our study was to evaluate the effects of three different nitrogen fertilizers (ammonium-nitrate, urea and calcium-nitrate) in combination with Microbion UNC bacterial fertilizer, on the plant available nutrients of soil and the nutrient uptake of horseradish *(Armoracia macrocarpa)*.

In the present study we summarize the effects of N fertilizers and bacterial fertilizer treatments on the available nutrient contents of soil.

MATERIALS AND METHODS

A field experiment was set up on the cultivation area of horseradish in Dombostanya in 2008. Dombostanya is located 15 km from Debrecen in Hungary. The soil of the experimental area had the following parameters: pH($CaCl_2$)= 7.47; Hu%= 2.87; $CaCO_3$=18.1%; K_A (plasticity index according to Arany) = 43; AL-P_2O_5= 144.6 mg kg^{-1}; AL-K_2O= 141.4 mg kg^{-1}; AL-Mg= 3156 mg kg^{-1}). The soil sufficiency levels of N and P were medium, K was poor. The bi-factorial trials were arranged in a randomized complete block design with three replications, applying different nitrogen fertilizers (ammonium-nitrate, urea and calcium-nitrate) with or without application of biofertilizer. The scheme of treatments applied can be seen in *Table 1*.

Table 1.

The scheme of treatments

Codes of treatments	N fertilizers	N dose (kg ha^{-1}) (as 116 kg N ha^{-1})	Microbion UNC (kg ha^{-1})
1.	0	0	0
2.	urea	249	0
3.	NH_4NO_3	331	0
4.	$Ca(NO_3)_2$	748	0
5.	0	0	2
6.	urea	249	2
7.	NH_4NO_3	331	2
8.	$Ca(NO_3)_2$	748	2

Doses of N fertilizers were divided, the first half was applied on 10th March, the second half was divided further into two parts and were applied on 10th June, and on 25th July. P and K were ensured as superphosphate (420 kg ha^{-1}) and potassium-sulphate (484 kg ha^{-1}), respectively, and were applied without dividing. Application of P and K nutrients were equalized for all treatments, 75.6 kg ha^{-1} P_2O_5 and 242 kg ha^{-1} K_2O, respectively.

The biofertilizer was Microbion UNC, which contains different microorganisms, Azotobacter vinelandii-B 1795, Bacillus megaterium-B1091, Clostridium pasteurianum, Azospirillum sp., Bacillus subtilis, Rhodobacter sp., Lactobacillus sp., Trichoderma reseei, Saccharomyces cerevisiae, Streptomyces sp., agents, vitamins synthetized by microorganisms, GM-8 corncob milling product and dried brewer's yeast. The bacterial fertilizer was mixed up and emitted with basic NPK fertilizers.

The experiment size of plots was 15m^2 (3m x 5m). Time of planting of horseradish was 11th april, and the picking time was 25th september 2008. After picking of horseradish representative soil samples were taken from each plot by auger up to 30 cm of depth. for determination of influences of different treatments. Soil samples were air dried and sieved (<2mm) for further analysis. Concentration of water soluble nitrogen forms (NO_3^--N, NH_4^+-N and total-N) were measured in 0.01 M $CaCl_2$ extracts with 1:10 soil:solution ratio (HOUBA et al., 1991) by autoanalyser (SKALAR Segment Flow Analyser). The soluble organic-N was calculated by the difference of soluble total-N and the sum of NO_3^--N and NH_4^+-N.

Concentration of phosphorus, potassium and magnesium in the soil taken up easily by plant were determined in ammonium lactate- acetic acid (AL) extract (EGNER et al., 1960). P was measured colorimetrically using the molybdenum blue colorimetric method, potassium was quantified by atomic emission spectrophotometry, while magnesium were determined by AAS method.

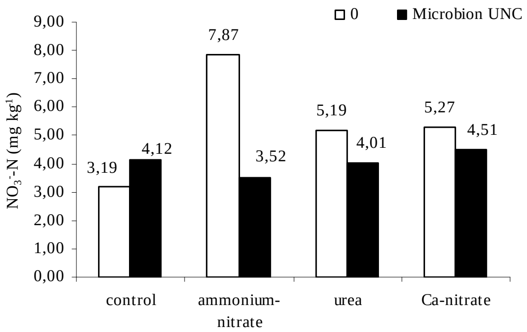
Analysis of variance was carried out on the data in order to provide a statistical comparison between the treatment means. The least significant difference (LSD) test was used to detect differences between means at probability level $P \leq 0.05$.

RESULTS AND DISCUSSION

Results of 0.01 M $CaCl_2$ soluble nitrogen forms

Concentrations of 0.01 M $CaCl_2$ soluble NO_3^- -N, NH_4^+-N, organic-N and total N are presented in *Figures 1.,2.,3.,4.* Regarding the N fertilizer treatments it can be stated that all N fertilizers proved to be effective and increased the NO_3^--N pool of the soil significantly compared to the control. We measured the highest values in the plot with NH_4NO_3 treatment. There was no significant difference between quantities of NO_3^--N of soil samples having urea and calcium-nitrate treatments.

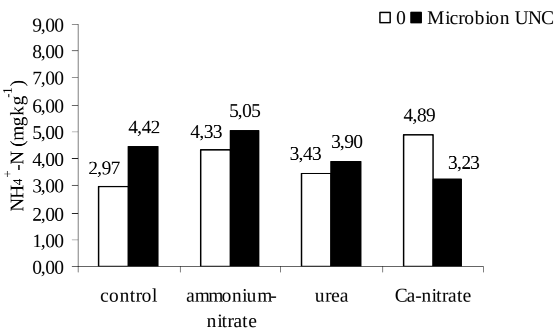
Figure 1: **0.01 M $CaCl_2$ extractable NO_3^--N content of soil as a function of different N fertilizer forms and Microbion UNC bacterial fertilizer**



$LSD_{5\%N}$=1.39 $SZD5_{\%UNC}$=0.99

Bacterial fertilizer increased (but not significantly) the amount of $CaCl_2$ soluble NO_3^--N of soil in the Microbion UNC treatment compared to the control (N_0), but in the case of other combined treatments, when artificial and bacterial fertilizer were applied together we measured lower NO_3^--N compared to the values measured in soil samples treated with N fertilizers only.

Figure. 2.: **0.01 M $CaCl_2$ extractable NH_4^+-N content of soil as a function of different N fertilizer forms and Microbion UNC bacterial fertilizer**



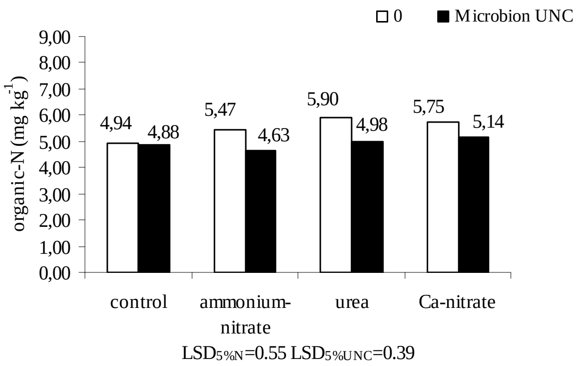
$LSD_{5\%N}$=1.0 $LSD_{5\%UNC}$=0.71

Our results show that the quantity of 0.01M $CaCl_2$ soluble NO_3^--N and NH_4^+-N were almost the same, the values of NO_3^- -N in some cases were a little bit higher than values of NH_4^+-N. The difference is the highest in the ammonium-nitrate treatment.

In plots treated with NH_4NO_3 and $Ca(NO_3)_2$ fertilizers we measured significantly higher NH_4^+-N values compared to the control, but in the case of urea treatment we did not measured notable different NH_4^+-N values.

Bacterial fertilizer supplement in most cases increased the $CaCl_2$ soluble NH_4^+-N content of soil compared to values with N fertilizer treatment. In the case of combined calcium-nitrate and Microbion UNC treatment we have found less NH_4^+-N content of soil samples compared to the value with calcium-nitrate treatment. The highest NH_4^+-N value was found in plot with treatment of ammonium-nitrate + Microbion UNC.

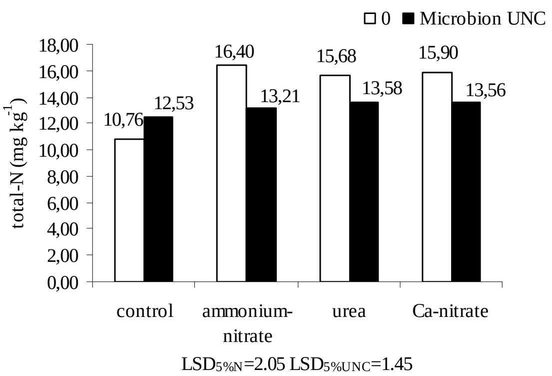
Figure. 3.: **0.01 M $CaCl_2$ extractable organic-N content of soil as a function of different N fertilizer forms and Microbion UNC bacterial fertilizer**



The quantity of soluble organic-N fraction was the same as the inorganic nitrogen fractions, and its values were balanced in the experiment. We measured only a slightly increased easily soluble organic-N fraction in plots having any nitrogen fertilizers, and we measured the highest values in the treatment of urea.

Microbion UNC fertilizer supplement decreased the soluble organic-N fraction of soil in all cases. This effect may be the result of the promoting of soil life, since with inoculation we may boost microbiological processes, such as mineralization, nitrification. Due to nitrification processes the quantities of soluble organic-N decreased and at the same time – as we measured - the inorganic nitrogen form, namely NH_4^+-N content increased.

Figure. 4.: **0.01 M $CaCl_2$ extractable total-N content of soil as a function of different N fertilizer forms and Microbion UNC bacterial fertilizer**



Measured data show that similarly to soluble inorganic and organic N fractions 0.01 M $CaCl_2$ soluble total-N were also higher when N fertilizers were applied, but these values was not depend on the form of the N fertilizer and were similar in all N treatments.

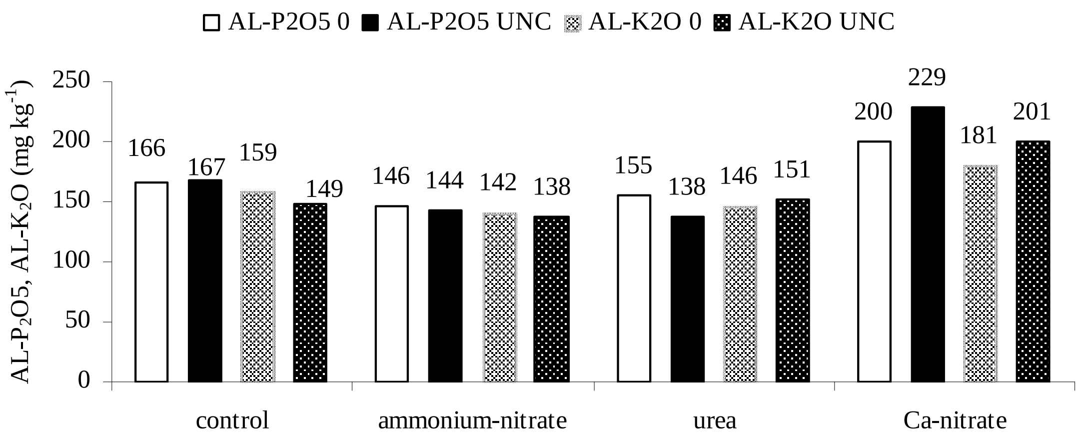
Bacterial fertilizer supplement also increased the amount of 0.01M $CaCl_2$ soluble total-N content of soil (Microbion UNC treatment) compared to the control (N_0), but in the case of combined treatments when both nitrogen and bacterial fertilizer were applied together, we measured lower total-N compared to the values measured in plots with N fertilizer treatments.

Results of AL-P_2O_5, AL-K_2O and AL-Mg values of soil

Concentrations of AL-P_2O_5, AL-K_2O of soil are presented in *Figure 5.*

On the basis of our results it can be concluded, that increased AL-P_2O_5 values were measured in the plots treated with calcium-nitrate compared to control. This increased values mentioned above appeared with or without bacterial fertilization also in the plots treated with calcium-nitrate. In the case of other two N fertilizer treatments, namely both ammonium-nitrate and urea treatments we measured lower AL-P_2O_5 values compared to control.

Figure. 5.: **AL-P_2O_5 and AL-K_2O contents of soil as a function of different N fertilizer forms and Microbion UNC bacterial fertilizer**



AL-P_2O_5 $LSD_{5\%N}$=11.2 AL-P_2O_5 $LSD_{5\%UNC}$=7.9

AL-K_2O $LSD_{5\%N}$=12.2 AL-K_2O $LSD_{5\%UNC}$=8.6

The effect of bacterial fertilizer on the AL-P_2O_5 values was different in case of different nitrogen fertilizers. While Microbion UNC supplement of calcium nitrate yielded the increase in AL-P_2O_5 values, till then supplement of urea yielded a decrease in AL-P_2O_5 values. Highest AL-P_2O_5 was measurable in plot treated with calcium-nitrate and Microbion UNC.

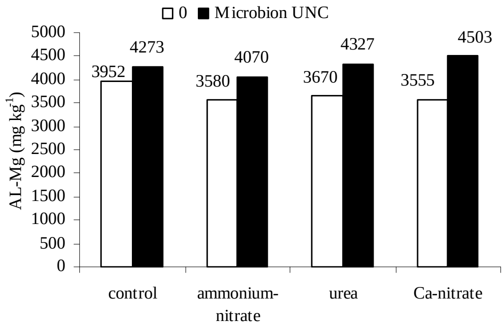
Soil measurements showed that increased AL-K_2O values (as in the case of AL-P_2O_5) appeared with or without bacterial fertilization also in the plots treated with calcium-nitrate. In the plots treated with ammonium-nitrate and urea we measured lower AL-K_2O content compared to the control.

The different influences of nitrogen fertilizers on the AL-P_2O_5 and AL-K_2O values may be a complex consequence of their effects on soil pH and their influence on the nutrient uptake of plant.

The effect of bacterium fertilizer on AL-K_2O values was also different in case of different nitrogen fertilizers. While Microbion UNC supplement of calcium-nitrate yielded increased AL-K_2O values, till then other cases AL-K_2O values did not differ. Highest AL- K_2O was measurable in plot treated with calcium-nitrate and Microbion UNC.

Concentrations of AL-Mg of soil are presented in *Figure 7.* According to our data all nitrogen fertilizers resulted in a significant decrease in the AL-Mg content of soil compared to the control. On the contrary, bacterial fertilizer supplement increased these values in any cases. We measured the highest AL-Mg content (4503 mg kg^{-1}) in plots treated with calcium-nitrate+Microbion UNC combined treatment. The influences of nitrogen fertilizers on the AL-Mg may be a consequence of their effects on the nutrient uptake of plant.

Figure. 6.: **AL-Mg content of soil as a function of different N fertilizer forms and Microbion UNC bacterial fertilizer**



LSD 5%N=281.3 $LSD_{5\%UNC}$=199.0

CONCLUSION

On the basis of our results it can be concluded that the amount of 0.01M $CaCl_2$ soluble inorganic nitrogen fractions, NO_3^--N and NH_4^+-N and the quantity of soluble organic-N were almost the same in the soil.

N fertilizers significantly increased all soluble N fractions of soil. The content of NO_3^--N increased to the greatest extent and the increase of organic N was the slightest. We measured the largest 0.01M $CaCl_2$ soluble NO_3^- -N and total-N contents in the plots treated with ammonium-nitrate, the largest NH_4^+-N in the plots treated with calcium-nitrate and the largest organic-N fraction in plots treated with urea.

Application of bacterial fertilizer (in Microbion UNC treatment) also increased both soluble inorganic nitrogen forms and total-N content of soil compared to the control. In the case of combined (application of artificial and bacterial fertilizer together) treatments we measured lower NO_3^--N, organic-N and total-N of plots compared to the values of plots having only nitrogen fertilizer treatments. On the contrary the 0.01M $CaCl_2$ soluble NH_4^+-N contents of soil in the plots with combined treatments in more cases were higher than that of values with artificial fertilizer treatments. In the case of combined, $Ca(NO_3)_2$+Microbion UNC treatment we measured lower NH_4^+-N values compared to appropriate $Ca(NO_3)_2$ treatment. The different effects of bacterial fertilizer on the soluble nitrogen forms may be the difference of the pH due to the application of artificial fertilizers.

As a function of calcium-nitrate fertilizer application increased AL-P_2O_5 and AL-K_2O values were measured in the plots compared to control.

The effects of bacterium fertilizer supplement were different on the AL-P_2O_5 and AL-K_2O values in case of different nitrogen fertilizers. Microbion UNC supplement of calcium nitrate yielded increased AL-P_2O_5 and AL-K_2O values.

All nitrogen fertilizers resulted in a significant decrease in the AL-Mg content of soil samples compared to the control. Nevertheless bacterial fertilizer supplement increased these values in any cases.

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