

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 (*Armoracia macrocarpa*) and available nutrients of the soil. Three different N fertilizers, ammonium-nitrate, urea and calcium-nitrate ($116 \text{ kg ha}^{-1} \text{ N}$) 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 analysis. The soil of the experimental area was chernozem with medium sufficiency level of N and P and poor level of K.

The main results are:

The amount of 0.01 M CaCl_2 soluble inorganic nitrogen fractions, $\text{NO}_3^- \text{-N}$ and $\text{NH}_4^+ \text{-N}$ and also the quantity of soluble organic-N were almost the same in the soil samples. N fertilizers significantly increased all the soluble N fractions. The amount of $\text{NO}_3^- \text{-N}$ increased to the greatest extent and the increase of organic N was the slightest. We measured the largest CaCl_2 soluble $\text{NO}_3^- \text{-N}$ and total-N contents in the plots treated with ammonium-nitrate, the largest $\text{NH}_4^+ \text{-N}$ 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 $\text{NO}_3^- \text{-N}$, 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 $\text{NH}_4^+ \text{-N}$ 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 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; Haynes, 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 solubilizing bacteria, cellulolytic microorganisms. They may promote plant growth and health by various means such as mineralization of nutritional elements, nodulation and nitrogen fixation (Malboobi et al., 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 (Kismányoky, 2009; Kádár, 2008; Berzsényi, 2009), 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; Makádi et al., 2007).

The aim of this 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: $\text{pH}(\text{CaCl}_2)=7.47$; $\text{Hu}\%=2.87$; $\text{CaCO}_3=18.1\%$; K_A (plasticity index according to Arany)=43; $\text{AL-P}_2\text{O}_5=144.6 \text{ mg kg}^{-1}$; $\text{AL-K}_2\text{O}=141.4 \text{ mg kg}^{-1}$; $\text{AL-Mg}=3156 \text{ mg kg}^{-1}$). 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 can be seen in Table 1.

Table 1.

The scheme of treatments			
Codes of treatments	N fertilizers	N dose (kg ha^{-1})	Microbion UNC (kg ha^{-1})
1. control (N_0)	0	0	0
2. U	urea	116	0
3. AN	NH_4NO_3	116	0
4. Ca-nitr.e	$\text{Ca}(\text{NO}_3)_2$	116	0
5. N_0 +UNC	0	0	2
6. U+UNC	urea	116	2
7. AN+UNC	NH_4NO_3	116	2
8. Ca-nitr+UNC	$\text{Ca}(\text{NO}_3)_2$	116	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, $75.6 \text{ kg ha}^{-1} \text{ P}_2\text{O}_5$ and $242 \text{ kg ha}^{-1} \text{ K}_2\text{O}$, 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 reesei*, *Saccharomyces cerevisiae*, *Streptomyces* sp., agents, vitamins synthesized 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 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.

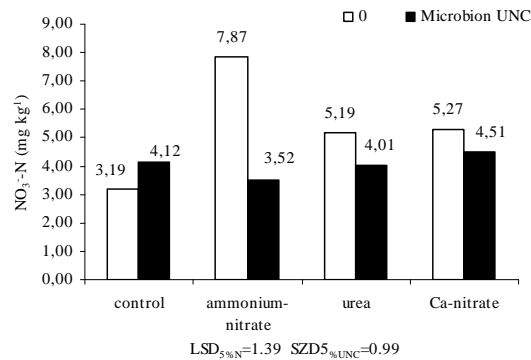
Analysis of variance was carried out on the data in order to provide a statistical comparison between the treatment means. The least significant difference (LSD) test was used to detect differences between means at probability level of $P \leq 0.05$.

RESULTS AND DISCUSSION

Results of 0.01 M CaCl₂ soluble nitrogen forms

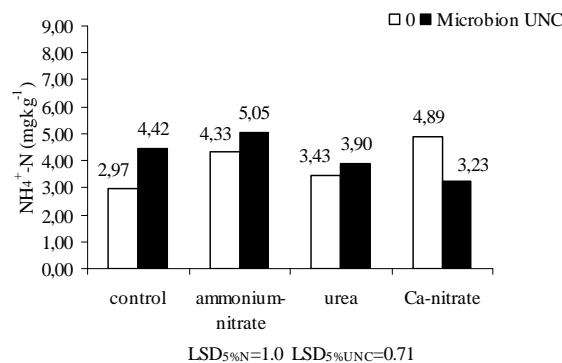
Concentrations of 0.01 M CaCl₂ soluble NO₃⁻-N, NH₄⁺-N, organic-N and total N are presented in *Figures 1.,2.,3.,4.* Regarding the N fertilizer treatments it can be stated that all N fertilizers proved to be effective and increased the NO₃⁻-N pool of the soil significantly compared to the control. The highest values were measured in the plot with NH₄NO₃ treatment.

Figure 1: 0.01 M CaCl₂ extractable NO₃⁻-N content of soil as a function of different N fertilizer forms and Microbion UNC bacterial fertilizer



Bacterial fertilizer increased (but not significantly) the amount of CaCl₂ soluble NO₃⁻-N of soil in the Microbion UNC treatment compared to the control (N₀), but in the case of other combined treatments, when artificial and bacterial fertilizer were applied together we measured lower NO₃⁻-N compared to the values measured in soil samples treated with N fertilizers only.

Figure. 2.: 0.01 M CaCl₂ extractable NH₄⁺-N content of soil as a function of different N fertilizer forms and Microbion UNC bacterial fertilizer

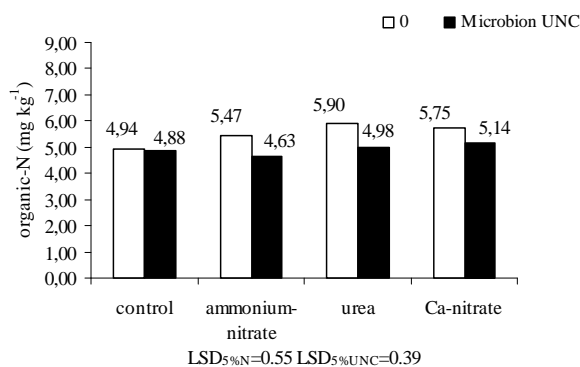


The results show that the quantity of 0.01M CaCl₂ soluble NO₃⁻-N and NH₄⁺-N were almost the same, the values of NO₃⁻-N in some cases were a little bit higher than values of NH₄⁺-N. The difference is the highest in the ammonium-nitrate treatment.

In plots treated with NH₄NO₃ and Ca(NO₃)₂ fertilizers we measured significantly higher NH₄⁺-N values compared to the control, but in the case of urea treatment we did not measured notable different NH₄⁺-N values.

Bacterial fertilizer supplement in most cases increased the CaCl₂ soluble NH₄⁺-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 NH₄⁺-N content of soil samples compared to the value with calcium-nitrate treatment. The highest NH₄⁺-N value was found in plot with treatment of ammonium-nitrate + Microbion UNC.

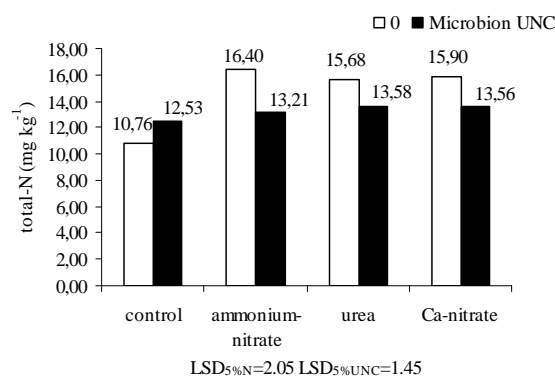
Figure. 3.: 0.01 M CaCl₂ extractable organic-N content of soil as a function of different N fertilizer forms and Microbion UNC bacterial fertilizer



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 soils in all cases. This effect may be the result of the promoting of soil life, since with inoculation microbiological processes such as mineralization, nitrification, etc. were boosted. 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



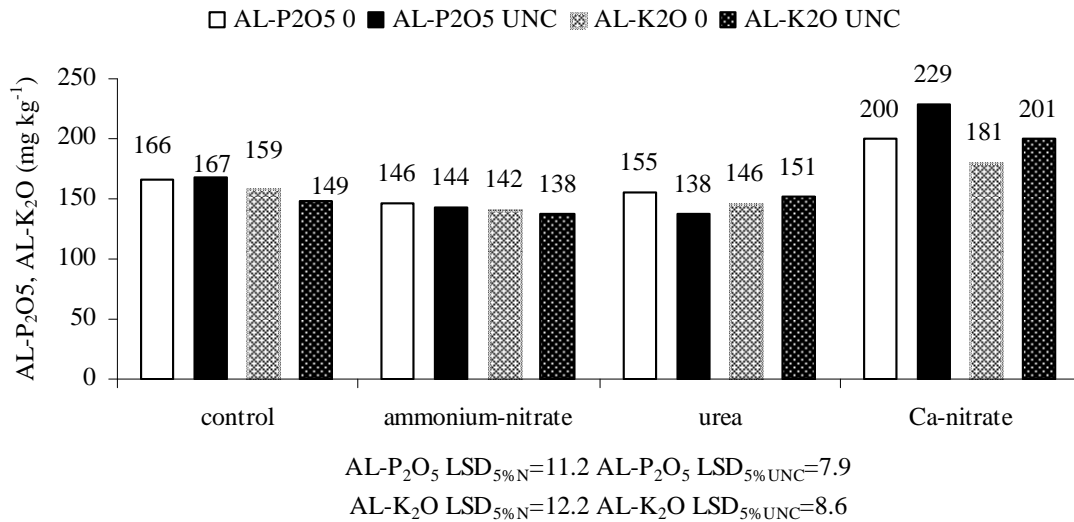
Measured data show that similarly to soluble inorganic and organic N fractions 0.01 M CaCl_2 soluble total-N were also higher when N fertilizers were applied, but these values was not depend on the form of the N fertilizer and were similar in all N treatments.

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, it was 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 soils are presented in Figure 5. On the basis of the 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 and without bacterial fertilization also in the plots treated with calcium-nitrate. In these treatments (Ca-nitrate and Ca-nitrate+Microbion UNC) the increase of AL- P_2O_5 values might be the consequence of the change of solubility because of the change of pH value. In the case of other two N fertilizer treatments, namely both ammonium-nitrate and urea it was measured lower AL- P_2O_5 values compared to control.

Figure. 5.: AL-P₂O₅ and AL-K₂O contents of soil as a function of different N fertilizer forms and Microbion UNC bacterial fertilizer



The effect of bacterial fertilizer on the AL-P₂O₅ values was not the same in case of different nitrogen fertilizers. While Microbion UNC supplement of calcium nitrate yielded the increase in AL-P₂O₅ values, till then supplement of urea yielded a decrease in AL-P₂O₅ values. Highest AL-P₂O₅ was measurable in plot treated with calcium-nitrate and Microbion UNC.

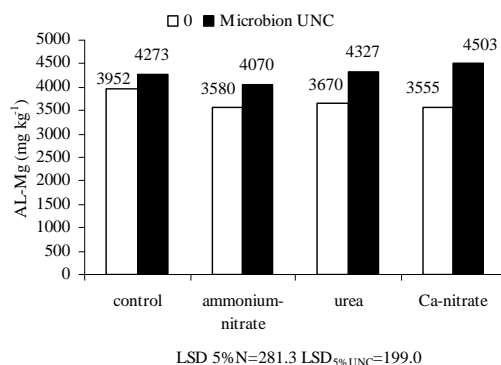
The soil measurements showed that increased AL-K₂O values (as in the case of AL-P₂O₅) appeared with and without bacterial fertilization also in the plots treated with calcium-nitrate. In the plots treated with ammonium-nitrate and urea it was measured lower AL-K₂O content compared to the control.

The different influences of nitrogen fertilizers on the AL-P₂O₅ and AL-K₂O values is 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-K₂O values was not also the same in case of different nitrogen fertilizers. While Microbion UNC supplement of calcium-nitrate yielded increased AL-K₂O values, till then other cases AL-K₂O values did not differ. Highest AL-K₂O 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



CONCLUSION

On the basis of the presented results it can be concluded that the amount of 0.01M CaCl₂ soluble inorganic nitrogen fractions, NO₃⁻-N and NH₄⁺-N and the quantity of soluble organic-N were almost the same in the soils.

N fertilizers significantly increased all soluble N fractions of soils. The content of NO₃⁻-N increased to the greatest extent and the increase of organic N was the slightest. The largest 0.01M CaCl₂ soluble NO₃⁻-N and total-N contents were measured in the plots treated with ammonium-nitrate, the highest NH₄⁺-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 soils compared to the control. In the case of combined (application of artificial and bacterial fertilizer together) treatments were measured lower NO₃⁻-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 CaCl₂ soluble NH₄⁺-N contents of soils in the plots with combined treatments were higher in more cases than that of values with artificial fertilizer treatments. In the case of combined, Ca(NO₃)₂+Microbion UNC treatment we measured lower NH₄⁺-N values compared to appropriate Ca(NO₃)₂ treatment. In the case of urea treatment it was not measured different NH₄⁺-N values with or without bacterial fertilizer supplement.

As a function of calcium-nitrate fertilizer application increased AL-P₂O₅ and AL-K₂O values were measured in the plots compared to control.

The effects of bacterium fertilizer supplement were different on the AL-P₂O₅ and AL-K₂O values in case of different nitrogen fertilizers. Microbion UNC supplement of calcium nitrate yielded increased AL-P₂O₅ and AL-K₂O 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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Különböző formájú nitrogén-műtrágyák és a Microbion UNC baktériumtrágya hatása a talaj felvehető tápelem-tartalmának változására