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\(_2\)O\(_5\) and AL-K\(_2\)O values were measured compared to control. Microbion UNC supplement of calcium nitrate yielded also increase in AL-P\(_2\)O\(_5\) and AL-K\(_2\)O 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 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, 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$_2$O$_5$= 144.6 mg kg$^{-1}$; AL-K$_2$O= 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.**

| 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 10$^{th}$ March, the second half was divided further into two parts and were applied on 10$^{th}$ June, and on 25$^{th}$ 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$_2$O$_5$ and 242 kg ha$^{-1}$ K$_2$O, respectively.

The biofertilizer was Microbion UNC, which contains different microorganisms, *Azotobacter vinelandii*-B1795, *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 11$^{th}$ april, and the picking time was 25$^{th}$ 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 ≤ 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. We measured the highest values in the plot with NH₄NO₃ treatment. There was no significant difference between quantities of NO₃⁻-N of soil samples having urea and calcium-nitrate treatments.

**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

Our 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.
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.

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$_2$O$_5$, AL-K$_2$O and AL-Mg values of soil**

Concentrations of AL-P$_2$O$_5$, AL-K$_2$O of soil are presented in Figure 5.

On the basis of our results it can be concluded, that increased AL-P$_2$O$_5$ 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$_2$O$_5$ values compared to control.
The effect of bacterial fertilizer on the AL-P$_2$O$_5$ values was different in case of different nitrogen fertilizers. While Microbion UNC supplement of calcium nitrate yielded the increase in AL-P$_2$O$_5$ values, till then supplement of urea yielded a decrease in AL-P$_2$O$_5$ values. Highest AL-P$_2$O$_5$ was measurable in plot treated with calcium-nitrate and Microbion UNC.

Soil measurements showed that increased AL-K$_2$O values (as in the case of AL-P$_2$O$_5$) 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$_2$O content compared to the control.

The different influences of nitrogen fertilizers on the AL-P$_2$O$_5$ and AL-K$_2$O 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$_2$O values was also different in case of different nitrogen fertilizers. While Microbion UNC supplement of calcium-nitrate yielded increased AL-K$_2$O values, till then other cases AL-K$_2$O values did not differ. Highest AL-K$_2$O 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 our 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 soil.

N fertilizers significantly increased all soluble N fractions of soil. The content of NO₃⁻-N increased to the greatest extent and the increase of organic N was the slightest. We measured the largest 0.01M CaCl₂ soluble NO₃⁻ -N and total-N contents in the plots treated with ammonium-nitrate, the largest 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 soil compared to the control. In the case of combined (application of artificial and bacterial fertilizer together) treatments we 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 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(NO₃)₂+Microbion UNC treatment we measured lower NH₄⁺-N values compared to appropriate Ca(NO₃)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-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.

REFERENCES

EGNER, H.-RIEHM, H.-MINGO,W.R., (1960): Untersuchungen über die chemische Bodenanalyse als Grundlage für die Beurteilung des Nährstoffzustandes der Böden. Kungl. Lantbruksögsk. Ann., Uppsala, 26. 199-215.

GHOST, B.C.-BHAT, R. (1998): Environmental hazards of nitrogen loading in wetland rice fields. Environ. Pollut. 102, 123–126.

GUTEZEIT, B.-FINK, M. (1999): Effect of cultivar and carrot date on nitrate content of carrot roots. Journal of Horticultural Science and Biotechnology, 74 (3): 297-300.

HAYNES, R.J. (1990): Active ion uptake and maintenance of cation anion balance: a critical examination of their role in regulating rhizosphere pH, Plant Soil 126, pp. 264–274.

HEGDE, D.M.-DWIVED, B.S.-SUDHAKARA, S.N. (1999): Biofertilizers for cereal production in India—a review. Indian J. Agric. Sci. 69, 73–83.

HINSINGER, P.-PLASSARD, C.-TANG, C.-JAILLARD, B. (2003): Origins of root-mediated pH changes in the rhizosphere and their responses to environmental constraints: a review, Plant Soil 248, pp. 43–59.

HOUBA, V.J.G.-JÁSZBERÉNYI, L.-LOCH, J. (1991): Application of 0,01 M CaCl₂ as a single extraction solution for evaluation of the nutritional status of Hungarian soils. Debreceni Agrártudományi Egyetem Tudományos Közleményei. 30. 85-89. p.

JALLOH, M.A.-CHEN, J.H.-ZHEN, F.R. (2009): Effect of different N fertilizer forms on antioxidant capacity and grain yield of rice growing under Cd stress Journal of Hazardous Materials 162. 1081-1085.

KÁDÁR, I.-MÁRTON, L.-NÉMETH, T.-SZEMES, I. (2007): Meszezés és mőtrágyázás hatása a talajra és növényre a 44 éves nyírlugosi tartamkísérletben Agrokémia és Talajtan, 56. 255-270.

KINCSES, S.-NAGY, P. T.-KREMPER R. (2008): A mő és baktériumintrágya hatása a növény-talaj rendszer makrotápelem-forgalmára tenyészdedénykíséretben. 50. Jubileumi Georgikon Napok Keszhely 09:25-25, 202-206.
LEWIS, O.A.M.-CHADWICK, S. (1983): An investigation into nitrogen assimilation in hydroponically-grown barley (Hordeum vulgare L. cv. Clipper) in response to nitrate, ammonium and mixed nitrate and ammonium nutrition, New Phytol. 95, 635–646.
MAIER, M.A.- MCLAUGHLIN, M.J.- HEAP, M.- BUTT M.- SMART, M.K. (2002): Effect of nitrogen source and calcium lime on soil pH and potato yield, leaf chemical composition and tuber cadmium concentration, J. Pl. Nutr. 25, 523–544.
MALBOOBI, MA.-BEHBAHANI, M.-MADANI, H. (2009) Performance evaluation of potent phosphate solubilizing bacteria in potato rhizosphere World Journal of Microbiology and Biotechnology 25. 1479-1484.
NYE, P.H. (1981): Changes of pH across the rhizosphere induced by roots, Plant Soil. 61. pp. 7–26.
VANCE, C.P. (1997): Enhanced agricultural sustainability through biological nitrogen fixation. In: Bio Fix of Nitrogen for Eco and Sustain Agric. Proc. NATO Adv Res. Work, Ponzan, Poland, 10–14 September 1996, Springer-Verlag, Berlin, Germany, pp. 179–185.
VESSEY, J.K. (2003): Plant growth promoting rhizobacteria as biofertilizers. Plant Soil 255, 571–586.