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Evaluation of 0.01 *M* $CaCl_2$ Extractable Nitrogen Forms in a Long-term Experiment

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A better knowledge of 0.01 *M* $CaCl_2$ extractable nitrogen forms may help improve the efficient use of fertilizers and organic manure. The amount of nitrate (NO_3^--N), ammonium (NH_4^+-N) and N-organic were measured in different treatments of Westsik's crop rotation experiment. The study aimed to optimize the nitrogen fertilizer recommendations for the Nyírség region. The fractionation method was based on a 0.01 *M* $CaCl_2$ extraction.

Introduction

Many studies (SCHNURER et al., 1985; ANDERSON & DOMSCH, 1989; ROSS & TATE, 1993) reported that soil microbial biomass and microbial activity are closely related to soil organic matter content. Microbial biomass correlates positively to the amounts of organic matter supplied over a long time, but it also responds to a single application of organic matter originating from post-harvest residues or organic manure (OCIO et al., 1991). NÉMETH et al. (1988) showed that the organic nitrogen fraction extracted by EUF is a reliable indication of mineralization during the growing season. According to NÉMETH (1996), mineralization and microbial immobilization of nitrogen are related to the soil, climatic conditions and nutrient amendments. In the case of nitrogen fertilization, the rate of organic matter mineralization increases, leading to a decrease in the content of easily decomposable organic matter (COLLINS et al., 1992; LOVELL & JARVIS, 1998; SZALÓKI-ZIMA & SZALÓKI, 2003). APPEL and MENGEL (1990) reported a close relationship between N-organic content and the N mineralization potential. They also showed that the nitrogen mineralization potential can be characterized with sufficient accuracy by using organic N-fractions. The $CaCl_2$ extraction was found to be more appropriate than EUF or the hot water extraction method. Organic fractions extracted by 0.01 *M* $CaCl_2$ solution were more closely related to nitrogen mineralization than the other two inorganic fractions. Similar results were reported by HOUBA et al. (1991), JÁSZBERÉNYI et al. (1994), LOCH and JÁSZBERÉNYI (1997) and NAGY et al. (2002).

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Organic nitrogen in the soil becomes available for plants after mineralization into inorganic forms that are related to microbial activity. Mineralization considerably depends on the decomposability and the C/N ratio of the soil organic matter. Generally proteins are more easily decomposed than other nitrogen compounds. According to GROOT and HOUBA (1995) the soluble organic nitrogen extracted with 0.01 *M* $CaCl_2$ solution is an index for the N-mineralization capacity of soils. In their experiment the organic nitrogen fraction extracted by 0.01 *M* $CaCl_2$ solution correlated well with the nitrogen uptake of ryegrass (APPEL & MENGEL, 1992). The 0.01 *M* $CaCl_2$ extractable (NH_4^+-N + NO_3^--N) predicts plant-available nitrogen in soils with a high inorganic N fraction; thus, 0.01 *M* $CaCl_2$ extractable (NH_4^+-N + NO_3^--N) may possibly be widely applicable as an index for plant-available nitrogen in fertilized soils.

Recent studies have shown that soluble organic nitrogen (SON) varied yearly between 8–20 kg/ha in coarse sand and 15–30 kg/ha in sandy loam. The minimum value was measured in winter; the maximum value in summer. MENGEL et al. (1999) reported 35–45 kg/ha 0.01 *M* $CaCl_2$ extractable organic nitrogen. Under continuous arable cropping MURPHY et al. (2000) measured 7–18 kg/ha SON after 8 years of grass lay in the 0–25 cm soil layer, which accounted for 33–60% of the total soluble nitrogen. Even higher SON was measured in a soil profile of 0–90 cm after ploughing up a long-term experiment on grassland. APPEL and MENGEL (1992) as well as KULCSÁR et al. (1997) found a correlation between SON and nitrogen mineralization, and suggested that SON extracted by 0.01 *M* $CaCl_2$ solution is a reliable indicator of organic N available for mineralization and plant uptake. MURPHY et al. (2000) gave account of similar results in loamy sand with KCl-extractable N-organic.

Chemical methods showing expressed correlations with actual field N mineralization include the EUF and 0.01 *M* $CaCl_2$ methods. DOU et al. (2000) found a strong correlation between the EUF extractable total nitrogen and nitrogen extractable by 0.01 *M* $CaCl_2$ solution. In their study, representative Florida soils were sampled and the electro-ultrafiltration (EUF) technique was used to measure the concentrations of total EUF-extractable nitrogen, NH_4^+-N and NO_3^--N. The nitrogen concentrations in the EUF extraction were greater than those measured with the 0.01 *M* $CaCl_2$ extraction method. The N-organic fraction, estimated by the EUF method, ranged from 4.4 to 40.8 mg/kg soil, equivalent to 10 to 91 kg/ha nitrogen for the 0–15 cm soil depth and was in positive correlation with the total soil nitrogen determined by the Kjeldahl method.

Nitrogen compounds extracted by electro-ultrafiltration (EUF) or $CaCl_2$ solution and their relationships with nitrogen mineralization were studied by MENGEL et al. (2000). Net mineralization was tested in Mitscherlich pot experiments with three treatments; (1) fallow soil without N fertilizer, (2) soil cultivated with ryegrass without N fertilizer, (3) soil cultivated with ryegrass with fertilizer. The highest proportion of nitrogen in the extracts were amino acids and peptides, amounting to approximately 60% and 40% of the total nitrogen extracted by $CaCl_2$ and EUF, resp. These methods measure soluble organic nitrogen compounds, which are expected to be closely related to the mineralization of organic nitrogen in the soil.

Although the organic fraction obtained by a 0.01 *M* $CaCl_2$ solution represents only about 1% of the total organic nitrogen, it still has a major impact on plant nutrition and nitrogen cycling. As a consequence of easy fractionation and low costs, the 0.01 *M* $CaCl_2$ extraction method is suitable for routine analysis and can be used widely for measuring plant available organic nitrogen. The introduction of this extraction method will possibly result in a more environment-friendly N fertilization without negative effects on the yield or quality of crops.

Material and Methods

Westsik's crop rotation experiment, established in 1929, includes 15 treatments. Crop rotations are divided into three series, with the exception of the one coded F-8, which has four. At the beginning the plot size was 2880 m², but due to mechanization it was reduced to 2700 m². The crop rotation experiment's total area is 12.4 hectares, as all series in each treatment are sown yearly. One of the main practical objectives of the crop rotation experiment was to measure the long-term effects of different organic manure and inorganic fertilizers on rye and potato production (WESTSIK, 1951). The experiment also makes it possible to study organic matter management under different applications of green, straw and farmyard manure and to study the ecological impact, as well as economical aspects of different management techniques.

Soil samples were collected in 2000, 2001 and 2002 from 45 different places/plots to study within plot variation. Samples were taken from a subplot of 300 m² and 5 elementary samples were combined, in order to obtain 9 representative samples on one plot (Fig. 1). No samples were collected from the borders of parcels. In addition to the sampling places soil temperature, water content and penetration resistance were measured in the 0–120 cm layer at 1 cm increments in the vegetation period of potato, just before flowering. The water holding capacity and water deficit were calculated for the 10–120 cm soil layer at 10 cm increments. Soil samples were taken by soil drill from the 0–20, 20–40, 40–60 cm depths. Samples were dried, ground and extracted with 0.01 *M* $CaCl_2$ (1:10 mass ratio, shaking for 2 h) and centrifuged at 48,000 g (HOUBA et al., 1986). Concentrations of NH_4^+ and NO_3^- were determined in the supernatant of centrifuged suspensions, using standard auto-

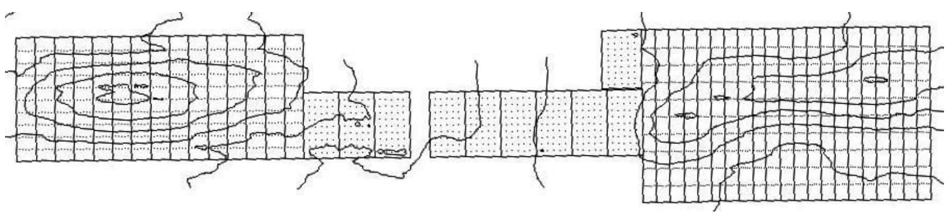

Fig. 1
Sampling places and contour lines in the treatments of Westsik's crop rotation experiment

analyser techniques (HOUBA et al., 1986). Extractable organic nitrogen was calculated as a difference between the total extractable nitrogen and mineral nitrogen determined with 0.01*M* $CaCl_2$ extract (HOUBA et al. 1986). Statistical methods applied include ANOVA and linear regression of SPSS statistics.

Results and Discussion

For ensuring the appropriate nutrition of crops, a balance needs to be found between achieving high yields and excellent quality, and simultaneously minimizing the leaching of nutrients. A possible approach is to determine the fertilizer requirement of a plant more precisely, as soil analytical methods can measure the soil nitrogen status and predict the mid-term availability of nitrogen more accurately. Recently, numerous methods have been developed for estimating the available nitrogen in soil, among them the organic nitrogen fraction. Organic material supply is important for maintaining favourable soil fertility and soil structure. The crop rotation experiment established by Vilmos Westsik in 1929 offers an excellent possibility for studying soil fertility management. Sources of organic matter include farmyard manure, mulches, harvest residues, green and root manures, which allow farmers to take advantage of the nitrogen fixing capacity of legumes.

Crop rotation F-1 receives no fertilizers and organic material, except litter, root and straw of cultivated plants incorporated into the soil. The fallow in this block is a green one, and weeds produced in the first series are ploughed into the soil. The F-2 represents green manure treatment, where the main crop is lupine, which is incorporated into the soil 4–5 weeks after flowering. The phosphorus and potassium fertilizers in this treatment are applied before lupine production. The F-3 represents lupine root manure treatment, where lupine is grown for grain and the total organic matter, with the exception of the grain, is incorporated into the soil (LAZÁNYI, 2003).

Blocks F-4 to F-7 represent straw manure treatments. In the F-4 block rye straw is applied as mulch. In crop rotation F-7 straw manure is fermented without nitrogen and in F-6 with nitrogen addition. The straw manure is incorporated into the soil 4–6 weeks before the sowing of rye. F-8 is the only block with 4 main crops, where lupine is grown twice in 4 years; once as a main crop produced for grain, and once as a second crop produced after rye and before potato, for green manure. In the F-9 block lupine is grown as a forage crop and harvested 2–3 weeks after flowering. Blocks F-10 and F-11 represent farmyard manure treatments without and with supplementary fertilizers, respectively. In crop rotation F-12 lupine is grown after a forage crop and sown in May. Blocks F-13, F-14 and F-15 also represent green manure treatments, in which lupine is grown as a second crop after rye and before potato. The F-15 block receives no supplementary fertilizers. The difference between blocks F-13 and F-14 is in the time of the incorporation of green manure.

The total nitrogen content of the soil in Westsik's crop rotation is between 0.02 and 0.2%, corresponding to a total amount of 0.5–3.5 t/ha in the top cultivated layer. A great part of the nitrogen derives from organic matter of floral, faunal and microbiological origin, as the level of the easily available organic nitrogen – consisting mainly of free amino acids and other small molecular weighted nitrogen compounds – increase in the proximity of the roots.

Figs. 2 and 3 demonstrate that the 0.01 *M* $CaCl_2$ extractable NO_3^--N is the largest pool for plant nutrition, while the proportion of 0.01 *M* $CaCl_2$ extractable N-organic ranged between 25 and 40% in the Westsik's crop rotation experiment treatments. The F-probe indicated a significant difference between the NO_3^--N contents of treatments. The highest NO_3^--N contents were found in treatments F-11, F-6 and F-8 (5.0, 5.3, 6.4 mg/kg soil, resp.). As a consequence of good mobility, the NO_3^--N content was very uniform in the 0–60 cm soil profile. Seventy-two years of fallow, lupine green and root manure treatments resulted in 1.7, 2.0, 2.3 mg/kg 0.01 *M* $CaCl_2$ extractable N-organic in the top 60 cm of the soil. The highest amount of N-organic was found in the F-5, F-6 straw manure and F-8 lupine green and root manure treatments, where rye and lupine were sown twice within a 4-year period.

The N-organic content was higher (3.4 mg/kg) in the top 20 cm layer, while this value was 2.2 mg/kg for the 40–60 cm depth. The soil NH_4^+-N content was the highest in the F-13, F-14 and F-15 treatments (Fig. 2).

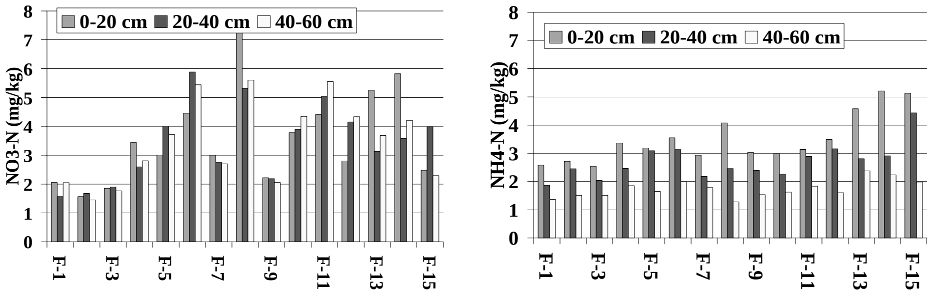

Fig. 2

0.01 *M* $CaCl_2$ extractable inorganic nitrogen forms in the treatments of Westsik's crop rotation experiment

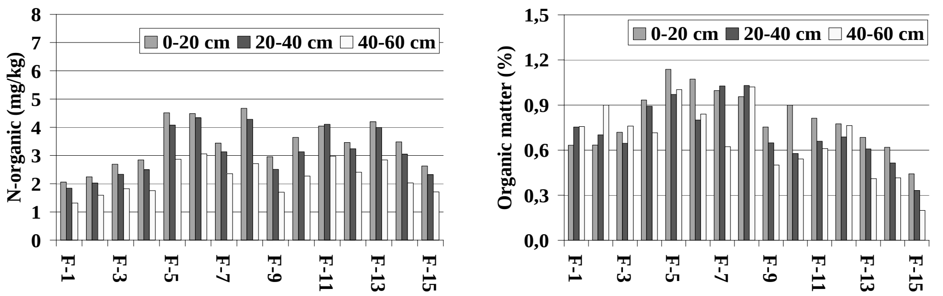

Fig. 3

0.01 *M* $CaCl_2$ extractable organic nitrogen and soil organic matter in the treatments of Westsik's crop rotation experiment

It is known that the microbial biomass not only plays an active part in the turnover, but is the most important labile pool of nitrogen and other nutrients in soil. Although the turnover of the soil microbial biomass is relatively high, the fluctuation in its values measured under natural conditions can hardly explain the expressed mineralization and immobilization processes observed at the same time, as no method is available for determining this pool directly.

0.01 *M* $CaCl_2$ extractable N-organic and NO_3^--N contents were lower at the top of the sand dunes and higher on the West side of the experimental plot, 200–250 m from the Érpatak stream (Figs. 4 and 5). NH_4^+-N demonstrated less variance. The organic matter is reconverted to mineral form by a wide variety of heterotrophic organisms, such as bacteria, fungi, and Actinomycetes. The drought index, soil NH_4^+-fixation, CEC, immobilization capacity, C:N ratio, soil temperature, depth of incorporation, size, as well as the amounts and quality of the incorporated material are the most important factors influencing nitrogen mineralization. In well-drained soils about 2% of the organic nitrogen is mineralized annually. Nitrification requires the presence of oxygen, and is highly sensitive to soil structure and water content. In aerobic soils the optimum water content is generally 50 to 67% of the

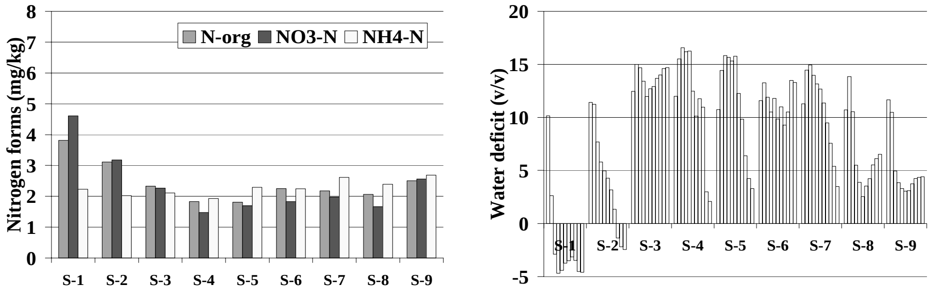

Fig. 4

0.01 *M* $CaCl_2$ extractable nitrogen forms and water deficit at different sampling places of the first sand dune (from F-1 to F-5 treatments)

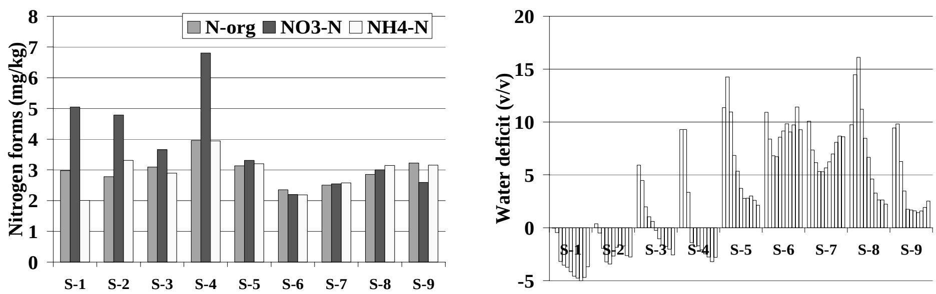

Fig. 5

0.01 *M* $CaCl_2$ extractable nitrogen forms and water deficit at different sampling places of the second sand dune (from F-9 to F-15 treatments) water holding capacity (GROOT & HOUBA, 1995). Waterlogging or flooding suppresses nitrification, and the ammonium produced tends to be absorbed by roots and soil colloids. Nitrification is temperature-sensitive and occurs mostly in the range 5 to 40 ^{o}C, with an optimum between 30 and 35 ^{o}C. In European countries soil temperature is usually the main influencing factor of mineralization. The oxidation of ammonium to nitrate is also temperature dependent. In Central and Eastern Europe limiting factors are often connected to soil moisture content, soil density, texture and tillage. The great difference in the 0.01 *M* $CaCl_2$ extractable N forms between the sampling places of Westsik's crop rotation experiment can also be explained by the water content of the soil on the sampling date.

In Hungary fertilizer recommendations are based on crop requirements, soil parameters, the drought index, nitrogen supply by crop residues, as well as the nitrogen and organic matter contents of the soil. The most important factors controlling nitrate leaching include the amount of rainfall, soil texture and structure, and the time, amount and form of nitrogen applied (CSATHÓ et al., 2005). Mineralization also influences nitrate losses (SZALÓKI-ZIMA & SZALÓKI, 2003). In a previous investigation a highly significant correlation was found between organic nitrogen extracted by 0.01 *M* $CaCl_2$ solution and potato yield (LAZÁNYI et al., 2002).

It has also been proven that soil organic nitrogen (N-organic) extracted by 0.01 *M* $CaCl_2$ solution is a reliable indicator of N-organic available for mineralization during the growing season. In any sustainable agricultural system the management of organic matter – and, more widely, nutrient management as a whole – is based on the total self-sufficiency of the farm.

Additionally, the entire cycle of organic matter production and decomposition takes place within the farm boundaries and makes the farm a biological system. The rate of metabolism and the organic matter cycle are characteristic features of such farms and define their activity for many years. When precise nitrogen fertilizer recommendations are required the 0.01 *M* $CaCl_2$ extraction method can supply useful information for environment-friendly, sustainable agriculture.

Summary

Soil nitrogen undergoes a series of chemical and biological transformations, which influence their availability to plants and the leaching losses. Methods for measuring various forms of nitrogen in soil are important in order to improve N management and to minimize losses of essential nutrients in soils.

In the present study 0.01 *M* $CaCl_2$ extraction was used to determine the concentrations of ammonium, nitrate and N-organic forms, as these are closely related to the N mineralization potential of the soil and play a major role in nitrogen availability to plants. Improving the precision of nitrogen requirement estimation is essential for increasing the efficiency and minimizing potential losses of nitrogen in agricultural production.

A better knowledge of the plant-availability of nitrogen may help improve the efficient use of fertilizers and organic manure. In the present study the amounts of N-NO_3, N-NH_4 and N organic were studied in Westsik's crop rotation experiment. The crop rotation experiment was established in 1929, and is the best known and most remarkable example of continuous production in Hungary, enabling the study of the long-term effects of organic manure treatment, the development of models and the prediction of the probable influences of different cropping systems on soil properties and crop yields in the Nyírség (sandy soil) region of Hungary.

The study aimed the optimization of the fertilizer recommendation system by considering the soil N-organic content as a measurement of site-specific mineralization potential. The 0.01 *M* $CaCl_2$ extraction method measures soluble organic N compounds that are related to the mineralization of organic nitrogen in the soil.

The introduction of this concept can possibly result in a more environment-friendly nitrogen fertilization, without negative effects on the yield or the quality of produced crops. Taking practicability and costs into consideration, the 0.01 *M* $CaCl_2$ extraction method is suitable for the routine analysis of measuring easily available organic nitrogen.

Key words: 0.01 *M* $CaCl_2$ extractable nitrogen, nitrogen fertilizer recommendation, sustainable agriculture

References

ANDERSON, T. & DOMSCH, K. H., 1989. Ratios of microbial biomass carbon to total organic carbon in arable soils. Soil. Biol. Biochem. **21.** 471–479.

APPEL, T. & MENGEL, K., 1990. Importance of organic nitrogen fractions in sandy soils, obtained by electro-ultrafiltration or $CaCl_2$ extraction, for nitrogen mineralization and nitrogen uptake of rape. Biology and Fertility of Soils. **10.** 97–101.

APPEL, T. & MENGEL, K., 1992. Nitrogen uptake of cereals grown on sandy soils as related to nitrogen fertilizers application and soil nitrogen fractions obtained by electro-ultrafiltration (EUF) and $CaCl_2$ extraction. European Journal of Agronomy. **1.** 1–9.

COLLINS, H. P., RASMUSSEN, P. E. & DOUGLAS, C. L. JR., 1992. Crop rotation and residue management effect on soil carbon and microbial dynamics. Soil Sci. Soc. Am. J. **56.** 783–788.

CSATHÓ, P. et al., 2005. Residual effects of nitrogen fertilization on brown forest soils in Western Hungary. (In Hungarian) Agrokémia és Talajtan. **54.** 59–76.

DOU, H., ALVA, A. K. & APPEL, T., 2000. An evaluation of plant-available soil nitrogen in selected sandy soils by Electro-Ultrafiltration, KCl, and $CaCl_2$ extraction methods. Biology and Fertility of Soils. **30.** 328–332.

GROOT, J. J. R. & HOUBA, V. J. G., 1995. A comparison of different indices for nitrogen mineralisation. Biology and Fertility of Soils. **19.** 1–9.

HOUBA V. J. G., JÁSZBERÉNYI, I. & LOCH, J., 1991. Application of 0,01 *M* $CaCl_2$ as a single extraction solution for evaluation of the nutritional status of Hungarian soils. Debreceni Agrártudományi Egyetem Tudományos Közleményei. **30.** 85–89.

HOUBA V. J. G. et al., 1986. Comparison of soil extractions by 0.01*M* $CaCl_2$, by EUF and by some conventional extraction procedures. Plant and Soil. **96.** 433–437.

JÁSZBERÉNYI, I., LOCH, J. & SARKADI, J., 1994. Experiences with 0,01 *M* calcium chloride as an extraction reagent for use as a soil testing procedure in Hungary. Commun. Soil Sci. and Plant Anal. **25.** 1771–1777.

JENKINSON, D. S. & RAYNER, J. H., 1977. The turnover of soil organic matter in some of the Rothamsted classical experiments. Soil. Sci. **123.** 298–305.

KULCSÁR, L. et al., 1997. Investigation of the soil N-fractions in special consideration of the N-fertilizer recommendation for sugarbeet. Poster, 11th World Fertilizer Congress, 7–13 September, 1997, Gent, Belgium.

LAZÁNYI, J., 2003. Results of Sustainable Production in Westsik's Crop Rotation Experiment. Westsik Vilmos Foundation for Rural Development. Nyíregyháza.

LAZÁNYI, J., LOCH, J. & JÁSZBERÉNYI, I., 2002. Analysis of 0.01 *M* $CaCl_2$ soluble organic nitrogen in the treatments of Westsik's crop rotation experiment. Agrokémia és Talajtan. **51.** 79–89.

LOCH, J. & JÁSZBERÉNYI, I., 1997. The 0.01 *M* $CaCl_2$ solution as a multi-element soil extractant – application and experiences in Hungary. In: Land Use and Soil Management. (Ed.: FILEP, GY.) 175–184. Rexpo Ltd. Debrecen.

LOVELL R. D. & JARVIS, S. C., 1998. Soil microbial biomass and activity in soil from different grassland management treatments stored under controlled conditions. Soil Biol. Biochem. **30.** 2077–2085.

MENGEL, K., SCHNEIDER, B. & KOSEGARTEN, H., 2000. Nitrogen compounds extracted by electroultrafiltration (EUF) or $CaCl_2$ solution and their relationships to nitrogen mineralization in soils. Journal of Plant Nutrition and Soil Science. **162.** 139–148.

MURPHY, D. V., MACDONALD, A. J. & STOCKDALE, E. A., 2000. Soluble organic nitrogen in agricultural soils. Biology and Fertility of Soils. **30.** 374–387.

NAGY, P. T., JÁSZBERÉNYI, I. & LOCH, J., 2002. The effect of fertilization on the 0.01 *M* calcium chloride extractable nitrogen forms in the long-term experiment at Nyírlugos. In.: Int. Conference on Long-term Experiments, Regional Production and Rural Development. (Eds.: LÁNG, I., LAZÁNYI, J. & NÉMETH, T.) (In Hungarian) **1.** 143–148.

NÉMETH, K. et al., 1988. Organic nitrogen compounds extracted from arable and forest soils by electro-ultrafiltration and recovery rates of amino acids. Biology and Fertility of Soils. **5.** 537–539.

NÉMETH, T., 1996. Organic Matter and Nitrogen Content of Soils. (In Hungarian) Research Institute for Soil Science and Agricultural Chemistry of the Hungarian Academy of Sciences. Budapest.

OCIO, J. A., MARTINEZ, J. & BROOKES, P. C., 1991. Contribution of straw-derived N to total microbial biomass N following incorporation of cereal straw to soil. Soil Biol. Biochem. **23.** 655–659.

ROSS D. J. & TATE, K. R., 1993. Microbial C and N, and respiratory activity in litter and soil of southern Beech (Nothofagus) forest: distribution and properties. Soil Biol. Biochem. **25.** 477–483.

SCHNURER, J., CLARHOLM, M. & ROSSWALL, T., 1985. Microbial biomass and activity in agricultural soil with different organic matter contents. Soil Biol. Biochem. **17.** 611–618.

SZALÓKI–ZIMA, I. & SZALÓKI, S., 2003. Nitrate leaching studies in long-term lysimeter and field experiments. (In Hungarian) Agrokémia és Talajtan. **52.** 35–52.

WESTSIK, V., 1951. Results of Crop Rotations on Sand. (In Hungarian) Mezőgazdasági Kiadó. Budapest.