Зборник Матице српске за природне науке / Proc. Nat. Sci, Matica Srpska Novi Sad, № 115, 41—49, 2008

UDC 631.416
DOI:10.2298/ZMSPN0815041K

Mirjana M. Kresović, Vlado Đ. Ličina, Svetlana B. Antić-Mladenović

University of Belgrade, Faculty of Agriculture, Nemanjina 6, Zemun, Serbia
e-mail: ktomi@eunet.yu

EVALUATION OF OPTIMAL TIME AND PARAMETERS FOR MEASURING POTENTIALY MINERALIZED NITROGEN IN SOIL

ABSTRACT: Our research was done on brown forest soil with long-term experiments and with a system of fertilizing which is in use for 40 years. Experiment variants with an increasing dose of nitrogen fertilizer were chosen for this research. Two experiments have been performed: experiment in pots supplied with ammonium nitrate labeled with a stable isotope ^{15}N (11.8%) and experiment in the field. The aim of the research was to establish which plant and soil parameters group (obtained in the controlled conditions and/or in the field) could be considered as reliable for evaluation of aerobic and anaerobic incubation and of the best time for estimation of potentially mineralized nitrogen in soil. According to the determined correlative dependence, it could be concluded that reliability of aerobic incubation should be estimated in October by plant and soil parameters from field, anaerobic incubation should be estimated in early spring (March) by plant and soil parameters, from controlled conditions (pots) and from field.

KEY WORDS: parameters, available nitrogen, aerobic, anaerobic, incubation

INTRODUCTION

All methods used today to determine the availability of soil nitrogen could be classified in three groups, namely: methods for determination of potentially mineralized nitrogen in soil, methods for determination of residual nitrogen, and the method of "A"-values (Goh and Haynes, 1986; Dhanke and Johnson, 1990; Bundy and Meisinger, 1994; Brown, 1996, Kresović, 1999, Bogdanović et. al., 2005).

Two groups of methods are used to estimate potential mineralized nitrogen in soil, namely: biological and chemical (biological and chemical indexes of nitrogen availability (Goh and Haynes, 1986; Brown, 1996).

Reliability of biological methods was established most often by using the plant parameters from controlled conditions when the parameters used were gained yield and adopted nitrogen (Keeney and Bremner, 1996; Ozus

and Hanway, 1966; Gasser and Kalembasa, 1976; Stevanovic, 1978). Reliability of before mentioned methods was rarely determined by using the field parameters (Robinson, 1968; Power, 1980; Hussian et al., 1994).

The purpose of these researches was to establish which of the used parameters of plants and soils (controlled conditions and/or field) could be considered reliable for estimation of aerobic and anaerobic incubation values. We also wanted to determine the most favorable time for estimation of potentially mineralized nitrogen (October, March, July).

MATERIAL AND METHODS

Researches were performed on a stationary experiment, on brown forest soil type.

The samples of soils were taken in October, March and July (0—30 cm depth) with experiment variance where the increasing doze of nitrogen fertilizer was applied.

a) Methods for determination of soil chemical properties

General chemical properties of investigated brown forest soil were determined in the soil samples taken in March, by using the following methods:

— Reaction of soil (pH-H_2O and 1 MKCl)

— Organic carbon and humus (Tjurin, modification Simakova, 1957)

— Total nitrogen (Semimikro Kjeldahl, Bremner, 1996)

— Available phosphorus and potassium (Al-method, Egner-Riehem, 1960).

b) Experiment in pots

Plastic pots containing 2 kg of soil were used to make the experiment in controlled conditions (greenhouse). The soil was taken three times in spring (in March) from the depth of 0—30 cm, from selected experiment variances in the field.

Before sowing, this soil was mixed with the following fertilizers: NH_4NO_3, KH_2PO_4 and KCl. Used ammonium nitrate was marked with a stable isotope ^{15}N (11.8%).

Vegetation experiment was made with two variants, PK and NPK. For PK variance, 50 mg of P_2O_5 and K_2O per kg of soil was used. For NPK variance, 50 mg of N, P_2O_5 and K_2O per kg was used. The experiment was applied with oats plants, with 10 plants in each pot, grown to the stage of jointing.

c) Experiment in field

The variances chosen for the experiment in field were those with increasing nitrogen fertilizer dose, as follows: control (Ø), N_1 P_2 K_2 (N_1 — 60, P_2 —

120, K_2 — 120 kg/ha); N_2 P_2 K_2 (N_2 — 90 kg/ha); N_3 P_2 K_2 (N_3 — 120 kg/ha); N_4P_2 K_2 (N_4 — 150 kg/ha). Each of mentioned experiment variances in the field was applied three times.

Wheat was raised in stationary experiment in these researches.

d) Methods for estimation of potentially mineralized nitrogen — aerobic and anaerobic incubation

Methods were applied to the soil samples taken from before mentioned experiment field variances in October, March and July.

Aerobic incubation was performed in accordance with proposed procedure (Bremner, 1965), while the anaerobic incubation was made with the procedure of Waring and Bremner, 1964, modification by Keeny (1982).

e) Method of isotopic labeled nitrogen (^{15}N)

During the application of ^{15}N isotope, the determination of percentage content of nitrogen isotope was made ($^{15}N/^{14}N$) by using the analyses of mass spectrometer. Isotope analysis was determined in accordance to Bremner procedure (1965).

f) Analysis of plant material

Nitrogen content in plants (oats and wheat) was determined by distillation method (Munsinger and McKinney, 1982).

g) Method of statistical analysis

A simple linear correlation analysis was applied for parameters of plants and soils in controlled conditions and in field, i.e. the methods for estimation of potentially mineralized soil nitrogen in all three times of taking samples.

This statistical analysis enabled us to determinate the value of correlation coefficient (Pirson) referring to the similarity degree, i.e. dependences of this compared value.

Based on the values of correlation coefficients, the percentage of correlative dependences for $r = 0.50—0.99$ was calculated, with significance at the probability level of 0.05—0.01 and for $r = 0.70$, with significance at the probability level of 0.01.

This calculation was made in order to use these data for the purpose of defining which group of applied parameters of plants and soils (pots and/or field) could be considered as the most appropriate for estimation on reliability of applied methods, as well as to establish the most reliable time to make those estimations (October, March or July).

RESULTS AND DISCUSSION

Chemical properties of investigated brown forest soil are shown in Table 1.

Tab. 1 — Chemical properties of investigated soil

Field variants	pH		Humus (%)	Total nitrogen (%)	C/N	Available	
	H_2O	nKCl				P_2O_5 (mg/100 g)	K_2O (mg/100 g)
Control	5.40	4.60	1.43	0.098	8.5	6.4	16.2
$N_1P_2K_2$	5.10	4.30	1.67	0.111	8.7	18.0	21.8
$N_2P_2K_2$	5.00	4.20	1.81	0.113	9.3	14.4	21.8
$N_3P_2K_2$	4.90	4.15	1.85	0.113	9.5	16.0	25.0
$N_4P_2K_2$	5.00	4.05	1.88	0.116	9.4	12.5	21.8

To establish which parameters of plants and soil might be considered reliable to evaluate the applied methods being one of the goals of these calculations, we have established the plant and soil parameters in the pots (controlled conditions) and in the field. Their values are shown in Tables 2, 3, 4, 5 and 6. Results are presented as mean values of three repetitions.

Tab. 2 — Parameters of plants in pot (g/pot)

Field variants	Yield		Total uptake of nitrogen NPK	Uptake of soil nitrogen NPK	Uptake of fertilizer nitrogen NPK	Total uptake of nitrogen PK
	NPK	PK				
Control	10.26	2.25	90.0	58.1	31.9	18.5
$N_1P_2K_2$	10.84	3.58	105.8	72.4	33.4	22.9
$N_2P_2K_2$	10.86	4.18	118.0	83.8	34.2	30.2
$N_3P_2K_2$	11.22	4.69	110.1	78.0	32.1	30.1
$N_4P_2K_2$	11.10	6.36	115.1	84.4	30.7	48.5

Tab. 3 — Parameters of plants in field (t/ha)

Field variants	Yield			% N grain	% N straw
	grain	straw	Total		
Control	0.95	1.21	2.16	1.239	0.209
$N_1P_2K_2$	3.90	6.08	9.99	1.463	0.261
$N_2P_2K_2$	4.38	6.30	10.68	2.015	0.272
$N_3P_2K_2$	4.97	6.21	11.17	2.172	0.424
$N_4P_2K_2$	5.13	6.77	11.90	2.359	0.426

Tab. 4 — Parameters of soil in field (kg/ha)

Field variants	Uptake of nitrogen (grain)	Uptake of nitrogen (straw)	Total uptake of nitrogen
Control	11.82	2.53	14.35
$N_1P_2K_2$	57.07	15.88	72.95
$N_2P_2K_2$	88.22	17.15	105.37
$N_3P_2K_2$	107.88	26.33	134.21
$N_4P_2K_2$	121.04	28.85	149.80

Taking into consideration the fact of most commonly used parameters and recommendations by S a p o s n i k o v (1973), evaluated parameters for applied methods were divided in two groups: parameters from plants and parameters from field that are further grouped in plant and soil parameters (Table 6).

Quantities of mineralized nitrogen by aerobic and anaerobic procedure are shown in Table 5 as mean values of three repetitions.

Tab. 5 — Quantities of mineralized nitrogen in aerobic procedure (ppm)

Field variants	Aerobic incubation			Anaerobic incubation		
	October	March	July	October	March	July
Control	15.7	24.7	50.6	16.2	14.8	18.6
$N_1P_2K_2$	21.5	24.2	48.1	17.7	18.0	19.0
$N_2P_2K_2$	21.8	32.2	62.8	14.6	17.7	19.4
$N_3P_2K_2$	28.3	30.6	103.4	21.6	19.7	20.4
$N_4P_2K_2$	26.0	27.8	54.3	17.0	20.1	18.7

Data on quantities of mineralized nitrogen obtained by aerobic and anaerobic procedure were compared with plant and soil parameters (pots and field) and the values of established correlation coefficients are presented in Table 6.

Tab. 6 — Correlation coefficient values between plant and soil parameters and nitrogen mineralized in aerobic and anaerobic procedure in brown forest soil

Parameters of plats of soil (pot)	Aerobic incubation			Anaerobic incubation		
	October	March	July	October	March	July
Yield (NPK)	0.75**	NS	NS	NS	0.70**	NS
Yield (PK)	0.74**	NS	NS	NS	0.90**	NS
% N in plants (NPK)	NS	0.56*	NS	NS	NS	NS
% N in plants (PK)	NS	NS	NS	NS	–0.54*	NS
Total uptake of nitrogen (NPK)	NS	0.54*	NS	NS	0.67**	NS
Uptake of soil nitrogen (NPK)	0.58*	0.57*	NS	NS	0.75**	NS
Uptake of fertilizer nitrogen (NPK)	NS	NS	NS	NS	NS	NS
Uptake of soil nitrogen/fertilizer	0.73**	NS	NS	NS	0.88**	NS
Soil nitrogen uptake (PK)	0.61**	NS	NS	NS	0.77**	NS
Parameters of plants and soil (field)						
Yield of grain	0.75**	NS	NS	NS	0.81**	NS
Yield of straw	0.70**	NS	0.53*	NS	0.76**	NS
Total yield	0.72**	NS	0.51*	NS	0.79**	NS
% N in grain	0.73**	NS	NS	NS	NS	NS
% N in straw	0.70**	NS	NS	NS	0.93**	NS
Uptake of nitrogen-grain	NS	NS	NS	NS	0.79**	NS
Uptake of nitrogen-straw	NS	NS	NS	NS	0.82**	NS
Total uptake of nitrogen	0.69**	NS	NS	NS	0.80**	NS

** — significant of probability level 0.01
* — significant of probability levels 0.05
NS — no statistic value

In March, the established correlation coefficients between the quantities of mineralized nitrogen obtained by aerobic procedure and the plant and soil

parameters from the experiment in pots were nearer to low than to average values. As for parameters of experiments in field, statistically significant correlation dependences were not found.

In July, there was no statistically significant correlation dependence for plant and soil parameters in pots while for parameters in field, the dependence was low.

In October, for plant and soil parameters from the pots, the statistically significant correlation dependence was not found for half of observed values, while for the remaining parameters it was high, with a high degree of statistical dependence.

Based on established values of correlation coefficients, a percentage of correlation dependences for given criteria was calculated (Table 7).

Tab. 7 — Correlation dependences (in percents) r = 0.50—0.90 (** and *) and for r ≥ 0.70** between the plants and soil parameters and nitrogen mineralized in aerobic procedure

Parameters	Percentage of correlation dependence for r = 0.50—0.90 (**, *)	Percentage of correlation dependences for r ≥ 0.70**
October		
Parameters of plants and soil in pots	55.5	33.3
Parameters of plants and soil in field	75.0	62.5
March		
Parameters of plants and soil in pots	33.3	0.0
Parameters of plants and soil in field	0.0	0.0
July		
Parameters of plants and soil in pots	0.0	0.0
Parameters of plants and soil in field	25.0	0.0

From obtained percentages of correlation dependences, it might be noted that the highest percentage was established in October. Thus, we can recommend applying the evaluation of potential mineralized nitrogen referring to the reliability of aerobic method in autumn (October), before the wheat seeding.

Based on established percentages of correlation dependences in October, it is obvious that the highest percentages were established for parameters from fields in both criteria. This percentage for the parameters from the pots, in both cases, was lower (55.5 band 33%). Thus, one can conclude that the evaluation of aerobic incubation value should be made by using the parameters from the field, being in conformity with the results of Robinson (1968). This is important having in mind that parameters from the field can be established by a much simpler way while it is more difficult to establish the parameters from the pots since it requires more work and use of expensive procedures (use of marked ^{15}N).

In anaerobic incubation, the same statistic analysis used in aerobic incubation was applied. The values of established correlation coefficients are presented in Table 6.

In March, the established correlation dependences between mineralized nitrogen obtained by anaerobic procedure and plant and soil parameters in pots

were mostly high, with a high degree of statistical significance, except for total adopted nitrogen (NPK) (0.67**) and for percentage of nitrogen in oats plants, where it was very low. A high correlation dependence between mineralized nitrogen obtained by mentioned procedure and plant and soil parameters from the field. In one case, it was very high (r = 0.93**—%N — straw) with a high degree of statistical significance.

In July and October, no statistically significant correlation dependences between plant and soil parameters (pots and field) and mineralized nitrogen were found when anaerobic procedure was applied.

Tab. 8 — Correlation dependences (in percents) r = 0.50—0.90 (** and *) and for r ≥ 0.70** between the plants and soil parameters and nitrogen mineralized in anaerobic procedure

Parameters	Percentage of correlation dependence for r = 0.50—0.90 (**, *)	Percentage of correlation dependences for r ≥ 0.70**
October		
Parameters of plants and soil in pots	0.0	0.0
Parameters of plants and soil in field	0.0	0.0
March		
Parameters of plants and soil in pots	77.78	55.5
Parameters of plants and soil in field	87.5	87.5
July		
Parameters of plants and soil in pots	0.0	0.0
Parameters of plants and soil in field	0.0	0.0

On the basis of the results shown in Table 8, it might be concluded that the highest percentages of correlation dependence for given criteria are found in March. Consequently, we can recommend making the evaluation of potential mineralized nitrogen by the anaerobic procedure in early spring (in March). In addition, from presented results in March, it is possible to note that the highest percentages of correlation dependences were established for parameters in the field for both given criteria. As for the parameters in pots, the percentages were lower. Taking into consideration the value of established percentages of correlation dependences, for a strict criterion (≥ 0.70**), we recommend to make the evaluation of anaerobic incubation value by using the parameters either in field or in pots.

CONCLUSION

On the basis of presented results, it can be concluded as follows:

— The evaluation of aerobic incubation reliability being a method for establishing potential mineralized nitrogen with conditions in brown forest soil should be performed in October.

— The evaluation of aerobic incubation should be made by using plant and soil parameters in the field.

— Evaluation of anaerobic incubation for given conditions should be performed in early spring (March).

— From used plant and soil parameters, to evaluate the value of anaerobic incubation, use equally the parameters from the field and from the pots.

REFERENCES

Bogdanović, D., Ubavić, M. I., Malešević, M. (2005): *Metode za utvrđivanje potreba biljaka za azotom.* Monografija: „Azot-agrohemijski, agrotehnički, fiziološki i ekološki aspekti". str. 151—183. Novi Sad.

Bremner, J. M. (1965): *Isotope-ratio analysis of nitrogen in nitrogen-15 tracer investigations*. In: "Methods of soil analysis" (C. A. Black, ed.), Part 2, Agronomy 9. Am. Soc. of Agron. P. 1256—1286 Madison, Wisconsin.

Bremner, J. M. (1996): *Nitrogen-total*. In: "Methods of soil analysis"; Part 3-Chemical methods, SSA book series 5; p. 1085—1121, am. soc. Agronomy, Madison, Wisconsin, USA.

Brown, N. M. (1996): *Evaluation of nitrogen availability indices*. Ph.D. thesis. Univ. of Illinois, USA.

Bundy, L. G. and Meisinger, J. J. (1994): *Nitrogen availability indices*. In: "Methods of soil analysis", part 2 — *Microbiological and biochemical properties*, SSA Book series 5, p. 951—979, Am. Sol. Agronomy. Madison, Visconsin, USA.

Dhanke, W. C. and Johnson, G. V. (1990): *Testing soils for available nitrogen.* In "Soil testing and plant analysis". p. 127—140. SSA Book series 3. Am. Soc. Agronomy, Medison, Wisconsin. USA.

Gasser, J. K. R. and Kalembasa, S. J. (1976): *The effects of leys and organic manures on the avalable-N in clay and sandy soils*. J. Soil Sci. Vol. 27, p. 237—249.

Goh, K. M. and Haynes, K. S. (1986): *Nitrogen and Agronomic Practice*. In: "Mineral nitrogen in the plant-soil system". (R. J. Haynes, ed.), Academic Press London, p. 379—442.

Hussain, F.; K. A. Malik and Naqui (1994): *A comparative study of different methods for obtaining an index of nitrogen availability in upcand soils*. Pak. J. Sci. Ind. Res. 37, 258—264.

Keeney, D. R. and Bremner, J. M. (1966): *Comparasion and evaluation of laboratory methods of obtaining an index of soil nitrogen availability*. Agron. J., vol. 58, p. 498—503.

Keeney, D. R. (1982): *Nitrogen availability indexes*. In: "Methods of soil analysis". Part 2nd Am. Soc. Agron. P. 711—733, Madison, Wisconsin.

Kresović, M. (1999): *Uporedna proučavanja metoda za ocenu pristupačnosti zemljišnog azota*. Doktorska disertacija, Beograd.

Musinger, R. A. and McKinney, R. (1982): *Modern Kjeldahl systes*. Am. Lab. 14, p. 76—79.

Ozous, T. and Hanway, J. J. (1966): *Comparisions of laboratory and greenhouse tests for nitrogen and phosphorus availability in soils*. Soil Sci. Soc. Am. Proc., Vol. 30, p. 224—228.

P o w e r, R. F. (1980): *Mineralizable soil nitrogen as an index of nitrogen availability to forest trees.* Soil Sci. Soc. Am. J. 44, p. 1314—1320.
R o b i n s o n, J. B. D. (1968): *A sample availabe soil nitrogen index: II. Field crop evaluation.* J. Soil Sci. 19, p. 280—290.
S a p o ž n j i k o v, N. A. (1973): *Metodi prognoza efektivnosti azotnih udobrenij.* Glava XII, 286—305, V knj.: „Azot v zemljedeljiji nečernozemnoj polsi". Izd-vo „Kolos", Lenjingrad.
S i m a k o v, V. N. (1957): *The use of phenylantaranicic acid in the determination of humus by tyurin's method.* Pochvoueudenie 15.
S m i t h, J. A. (1966): *An evaluation of nitrogen soil test methods for Ontario soils.* Can. J. Soil Sci. 46, p. 185—196.
S t e v a n o v i ć, D. (1978): *Sadržaj nekih oblika azota u gajnjači i njihov uticaj na efikasnost azotnih đubriva.* Agrohemija, N° 7—8, st. 307—315.
W a r i n g, S. A. and B r e m n e r, J. M. (1964): *Ammonium production in soil under water logged conditions as an index of N availability*. Nature, 201 p. 951—942. London.

УТВРЂИВАЊЕ ОПТИМАЛНОГ ВРЕМЕНА И ПАРАМЕТАРА ЗА ОЦЕНУ ПОТЕНЦИЈАЛНО МИНЕРАЛИЗУЈУЋЕГ АЗОТА ЗЕМЉИШТА

Мирјана М. Кресовић, Владо Ђ. Личина, Светлана Б. Антић-Младеновић

Универзитет у Београду, Пољопривредни факултет,
Немањина 6, Земун, Србија

Резиме

Истраживања су обављена на земљишту типа гајњача (околина Младеновца) на којем је постављен стационарни оглед са одређеним системом ђубрења. За ова истраживања одабране су варијанте огледа из поља где је примењена растућа доза азота ђубрива. Циљ истраживања је био да се утврди који се параметри биљака и земљишта (контролисани услови и/или поље) могу сматрати поузданима за оцену вредности аеробне и анаеробне инкубације, као и да се утврди најповољније време за ту оцену. Да би се остварио постављени циљ истраживања, изведена су два огледа: оглед у судовима (контролисани услови) где је примењен амонијум нитрат који је био обележен стабилним изотопом ^{15}N (11,8%) и оглед у пољу. Да би се утврдило оптимално време за оцену поузданости аеробне и анаеробне инкубације, са стационарног огледа су узорци земље узимани у октобру, марту и јулу. На основу утврђених корелативних зависности, за услове који су владали у испитиваној гајњачи, оцену поузданости аеробне методе треба доносити у октобру коришћењем параметара биљака и земљишта из поља. Оцену поузданости анаеробне методе треба утврђивати у рано пролеће (март), при чему се за ту оцену могу равноправно користити како параметри биљака и земљишта из поља, тако и из судова (контролисани услови).