Journal of the Serbian Chemical Society
JSCS@tmf.bg.ac.yu • www.shd.org.rs/JSCS
www.shd.org.rs

J. Serb. Chem. Soc. 74 (1) 93–102 (2009) UDC 546.17–032.6+631.415:66.095.81:66.091
JSCS–3812 *Original scientific paper*

Specific transformations of mineral forms of nitrogen in acid soils

MIRJANA KRESOVIĆ[1], MIODRAG JAKOVLJEVIĆ[1], SRDJAN BLAGOJEVIĆ[1*#]
and SRBOLJUB MAKSIMOVIĆ[2]

[1]*Faculty of Agriculture, Nemanjina 6, 11080 Zemun, Belgrade and* [2]*Institute of soil science, Teodora Drajzera 7, 11000 Belgrade*

(Received 21 March, revised 30 June 2008)

Abstract: Investigations were performed on soils of different acidity, ranging in the pH interval 4.65–5.80 (in water). Changes of the mineral nitrogen forms in the examined soils were studied by applying short-term incubation experiments performed under aerobic conditions, with a humidity of 30 % and a temperature of 20 °C, both with and without the addition of 100 and 300 ppm NH_4–N. The results of the incubation experiments showed that retarded nitrification was present in all the examined soils. Increased and toxic quantities of nitrites (35.7 ppm) were formed during the incubation, which remained in the soil solution for several days, and even weeks, in spite of favorable conditions of moisture, aeration and temperature for the development of the process of chemoautotrophic nitrification. Decelerated chemoautotrophic nitrification was the source of the occurrence of nitrite in the examined less acid soil (soil 1), while in soils of higher acidity (soils 2 and 3) after addition of 100 and 300 ppm NH_4–N, nitrite occurred due to chemical denitrification (chemodenitrification). Nitrites formed in the process of chemodenitrification underwent spontaneous chemical oxidation resulting in nitrate formation (chemical nitrification). The content of mineral nitrogen (NH_4 + NO_3 + NO_2–N) decreased during the incubation period, proving gaseous losses from the examined soils. Application of lower doses of nitrogen fertilizers could decrease nitrogen losses by denitrification as well as the occurrence of nitrite in toxic quantities in the investigated pseudogley soil.

Keywords: incubation; mineral nitrogen; nitrification; denitrification; chemodenitrification.

INTRODUCTION

The processes of mineralization and nitrification represent integral parts of the cycle of nitrogen circulation in soil.

---

[*] Corresponding author. E-mail: sblagoje@eunet.yu
[#] Serbian Chemical Society member.

doi: 10.2298/JSC0901093K

Nitrogen mineralization in soil is a process in which organic nitrogen compounds are decomposed releasing non-organic nitrogen (NH_4–N). Non-specific microorganisms (bacteria, actinomycetes, fungi) participate in the mineralization process. As for the ambient conditions involved in the mineralization process, the content of organic matter in the soil has the biggest influence while the influence of soil acidity is a little smaller, bearing in mind that this process also occurs in an acidic environment. This is because fungi, being acidophilic microorganisms, play a significant role in the transformation of organic nitrogen compounds. In neutral and alkaline soils, mineralization develops due to the presence of bacteria.[1]

The largest differences in the nitrogen circulation cycle in acid soils refer to the nitrification process.

Biological nitrification is an extremely specific process in soil, with the participation of specific microorganisms from the group of real bacteria. The process of biological nitrification is performed in two phases, namely: ammonia oxidation (nitrition) with the participation of *Nitrosomonas*:

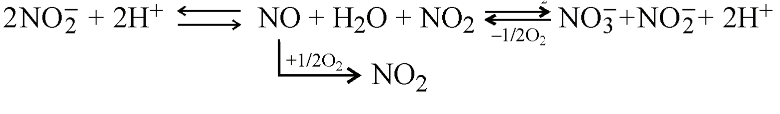
$$NH_4^+ + 1/2O_2 \xrightarrow{6e^-} NO_2^- + 2H^+ + H_2O$$

and nitrite oxidation (nitration), with the participation of *Nitrobacter*:

$$NO_2^- + 1/2O_2 \xrightarrow{2e^-} NO_3^-$$

Nitrification bacteria are sensitive to the influence of the external environment, where the pH value of the soil represents one of the limiting factors. Thus, a pH value of 4.5 (water) is taken as the lower threshold for the activity of autotrophic nitrificators.[2] In addition to this, high levels of ammonium nitrogen can also inhibit the activity of *Nitrobacter,* resulting in an accumulation of NO_2–N in soil.[3,4] Nitrification in acid soils has some specific features differing to that in neutral and alkaline soils. Namely, in acid soils, due to the higher sensitivity of nitrate bacteria to the low pH value of the soil, nitrification is often significantly slower resulting in nitrite accumulation.[5] Thus, nitrites in acid soils are accumulated, their quantity is rather small (several ppm) but sufficient to have a toxic influence on plants and microorganisms, especially if higher concentrations exist for a longer period.

In very acid soils (pH lower than 4.5 in 1.0 M KCl), a chemical oxidation of nitrites into nitrates (chemical nitrification) is possible, whereby the nitrite nitrogen acts as a self-donor and acceptor of electrons.[6,7] The process of chemical nitrification can be presented by the following reaction:



$$2NO_2^- + 2H^+ \leftrightarrows NO + H_2O + NO_2 \underset{-1/2O_2}{\overset{+1/2O_2}{\rightleftarrows}} NO_3^- + NO_2^- + 2H^+$$

$$NO \xrightarrow{+1/2O_2} NO_2$$

Bearing in mind the fact that nitrites are formed in the first phase of nitrification (nitrition), they can be found in the soil as a result of the denitrification process. Denitrification process in a wider sense means gaseous nitrogen losses from the soil in a microbiological or chemical way where volatilization is excluded.

The process of biological denitrification can be presented as:

$$NO_3^{(+5)} \rightarrow NO_2^{(+3)} \rightarrow NO^{(+2)} \rightarrow N_2O^{(+1)} \rightarrow N_2{}^{(0)}$$

In this process, nitrites occur as intermediate products since the denitrification bacteria reduce NO_3 into NO_2 faster than NO_2 into N_2[8] and the final products of this process are gaseous nitrogen and its oxides. This process occurs in the absence of oxygen, in the presence of organic matter and by neutral or alkaline reactions.

The process of biological denitrification cannot explain all the gaseous losses from soils, especially when they occur in completely aerated soil. It was concluded that these losses occur by chemodenitrification and in addition to all, when there is an intense accumulation of nitrites and when the pH value of the soil is lower than 5.5 (water), the final products of this process are NO, N_2O or N_2 and the nitrites appear as an intermediate product. The appearance of the aforementioned gases in a completely sterilized soil is confirmation of the chemodenitrification process.[9] The chemodenitrification process occurs by one of the following reactions:

1. Self-decomposition of nitric acid:

$$2HNO_2 \rightarrow NO + NO_2 + H_2O$$
$$3HNO_2 \rightarrow 2NO + HNO_3 + H_2O$$

2. Formation of molecular nitrogen in reactions of NO_2 with ammonium salts and amino acids:

$$NH_4 + HNO_2 \rightarrow N_2 + 2H_2O + H$$
$$R\text{–}NH_2 + 2HNO_2 \rightarrow N_2 + R\text{–}OH + H_2O$$

3. Formation of NO and N_2O from accumulated nitrites with participation of Fe^{2+} and Mn^{2+}:

$$NO_2^- + 2H^+ + Fe^{2+} + Mn^{2+} \rightarrow Fe^{3+} + Mn^{3+} + NO + H_2O$$
$$2NO + 2H^+ + Fe^{2+} + Mn^{2+} \rightarrow Fe^{3+} + Mn^{3+} + N_2O + H_2O$$

Therefore, the nitrites in the soil are dominantly formed in two processes, namely in the process of nitrification and in the process of denitrification. In acid soils, nitrites often accumulate as a result of retarded biological nitrification[10] and chemodenitrification,[9] as well as in the presence of higher quantity of ammonium nitrogen in soil.[11] Formed nitrites in acid soils in any of the aforementioned ways can remain for a longer or shorter period. The presence of increased nitrite quantities in the soil causes temporary chlorosis of plants and even their complete destruction.[5,12] Nitrites can also have toxic effects to humans and animals.[13,14] The quantities higher than 5 ppm in acid soils are considered as toxic and 10 ppm in neutral and alkaline soils, respectfully.[5]

The scope of these researches resulted from the fact that in Serbia there is the area of around 500,000 hectares of pseudogley type soil,[15] representing a significant part of the total cultivable area. The basic chemical property of pseudogley soil is that it belongs to the category of acid soils with typical excesses of soluble Al and Mn, which have harmful effects on cultivated plants.[16] Additionally, the occurrence of toxic quantities of nitrite nitrogen is possible, especially when larger quantities of fertilizer are applied. The purpose of this investigation was to determine in which quantities and under what conditions nitrites are accumulated in pseudogley soil, bearing in mind the fact that they can be toxic to plants.

EXPERIMENTAL

The experiments were performed on pseudogley soils taken from three localities near the city of Šabac. The soil samples were taken from a depth of 0–20 cm and then air-dried, triturated and sieved through a 2 mm sieve. The incubation experiments were performed and the chemical properties of the experimental soil established with samples prepared in this manner.

In order to observe nitrogen specific transformations in acid soils, three pseudogley soils of different acidity were chosen and incubation experiment under optimum moisture and temperature conditions was performed using high (100 ppm NH_4–N) and very high nitrogen doses (300 ppm NH_4–N). These doses are often used in inappropriate application of mineral fertilizers in fields, having negative effects on the plants, especially in acid soils.

Incubation experiments

The tested soils were submitted to short-term incubation experiments by a proposed procedure.[17] The following treatments: soil + water, soil + 100 ppm NH_4–N and soil + 300 ppm NH_4–N were applied. The experiments were performed with 30 % soil moisture at 20 °C. Thus, 10 g of soil samples were put in glass jars having a volume of 150 ml and then 3 ml of distilled water and 3 ml of an $(NH_4)_2SO_4$ solution containing 100 and 300 ppm NH_4–N were added. The jars were closed with a thin plastic foil and thermostated at 20 °C. After the required incubation period (5, 8, 12, 15, 19 or 22 days), the jars were opened and 100 ml of a 2.0 M KCl solution added. The jars were then shaken for 1 h and the contents filtered.

The contents of NH_4, NO_3 and NO_2–N were determined in the extracts by the distillation method with hot water vapor. MgO as an alkaline agent and Devarda alloy were used in this method to reduce nitrate to ammonium. It was necessary to perform three distillations to determine NH_4, NO_3 and NO_2–N. In the first distillation of the extract, NH_4–N was determined when the MgO base was added and in the second distillation, NO_3 + NO_2–N was determined when the Devarda alloy was added. A fresh quantity of extract was taken for the third distillation, MgO was added as in the first distillation and the NH_4–N content was again determined, thus enabling the accuracy of the first distillation to be controlled. The remaining extract was cooled and a 0.20 M solution of sulfamine acid was added in order to destroy the nitrites. Devarda alloy was afterwards added, enabling the determination of the content of

NO_3–N. The content of NO_2 was determined from the difference between confirmed nitrogen content in the second distillation (NO_3 + NO_2–N) and the content of NO_3 determined in the third distillation.[18]

Determination of the basic chemical properties of examined soils

The pH value of the soil was determined by an Iskra pH meter using a glass (indicator) and a calomel (reference) electrode in suspensions: soil:water = 1:2.5 and soil:1 M KCl = 1:2.5.

The humus content was determined volumetrically after oxidation with excess 0.40 M $K_2Cr_2O_7$ in an acid environment (H_2SO_4). The $K_2Cr_2O_7$ remaining after the oxidation of the organic carbon in the soil was determined by the oxidation–reduction reaction with 0.10 M $(NH_4)_2Fe(SO_4)_2$ using phenilanthranilic acid as the indicator. The $K_2Cr_2O_7$ solution was standardized volumetrically with $KMnO_4$, the molarity of which had been determined using pure oxalic acid as the standard substance.

The method for determination of the content of total nitrogen in the soil consisted of two steps: (1) sample digestion in order to transform the organic nitrogen into NH_4–N and (2) determination of NH_4–N in the solution after digestion. The digestion was performed by gradual heating to a temperature of 380 °C with p.a. H_2SO_4 and by adding a catalyst mixture (K_2SO_4:$CuSO_4$:Se = 100:10:1) in a period of 4 h. The NH_4–N in the obtained digest was determined by catching the NH_3 released during the digest distillation with hot water vapor in the presence of excess NaOH. The released NH_4 passed through a cooler and was taken in a given volume of boric acid. Then, titration of the ammonium borate was performed with 0.010 M H_2SO_4.

Content of the mineral forms of nitrogen (NH_4, NO_3 and NO_2–N) before and after incubation were determined by the distillation method after extraction with 2.0 M KCl (100 ml), as explained in the part concerning the procedure for performing the incubation experiments.

RESULTS AND DISCUSSION

The chemical properties of the examined soils are given in Table I.

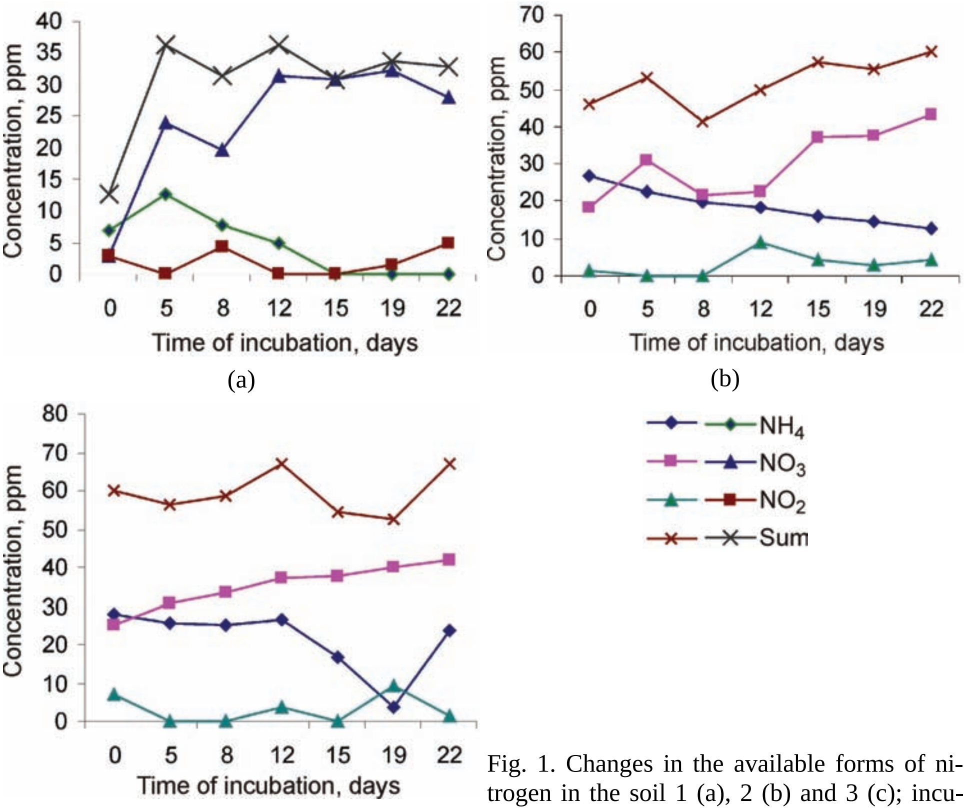
TABLE I. Basic chemical properties of the examined soils

Soil	pH		Total nitrogen	Humus	Organic C	Available N / ppm			
	Water	*n*KCl	%	%	%	NH_4–N	NO_3–N	NO_2–N	Sum
Soil 1	5.80	4.80	0.11	1.60	0.93	7.0	2.8	2.8	12.6
Soil 2	4.90	3.45	0.12	1.44	0.84	26.6	18.2	1.4	46.2
Soil 3	4.65	3.20	0.13	1.80	1.04	28.0	25.2	7.0	60.2

Examined soils can be classified into the group of acid ones, whereby soil 1 was acid while soils 2 and 3 belong to the category of very acid. All three examined soils have a low content of humus and total nitrogen. The content of available nitrogen at the time of sampling of soil 1 was low and of soils 2 and 3 was high.

The results of the incubation experiments for the examined soils with only water added (soil + water) are shown in Fig. 1.

Initial content of available nitrogen in soil 1 was the lowest (12.5 ppm), while in other two soils had much higher amounts (soil 2 – 46.2 ppm and soil 3 – – 60.2 ppm).



(a)

(b)

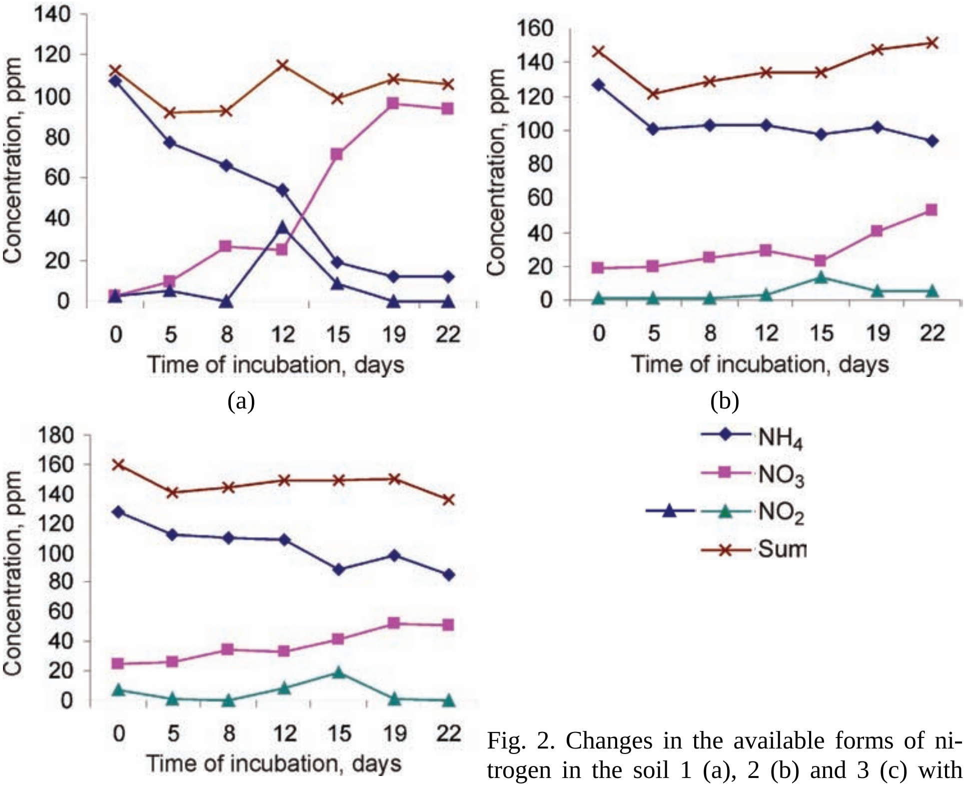
(c)

Fig. 1. Changes in the available forms of nitrogen in the soil 1 (a), 2 (b) and 3 (c); incubation in soil+water.

Taking into consideration the initial contents of available nitrogen in soil 1, the most intense nitrogen mineralization was created in a short time period (from initial 12.6 to 36.4 ppm after 5 days). During further incubation, the quantities of mineralized nitrogen showed a trend of either increasing or decreasing. This can be explained by the periodic occurrence of denitrification when some of the nitrogen disappears, while in the mineralization process it is generated. Nitrogen transformation in this soil was characterized by fixation of newly formed NH_4–N, as well as its fast nitrification. NH_4–N had disappeared by the end of experiment, but increased quantities of nitrogen nitrite forms were generated after 8 days and at the end of incubation period, day 22, had reached a value of 4.9 ppm. This can be connected with the processes of nitrification and denitrification, in which nitrites are formed as intermediate products.

Soil 2 was more acid (pH 4.90 in water) compared to soil 1 (pH 5.80), so that nitrogen mineralization was significantly retarded in this soil. The retardation was even more pronounced in soil 3, which had the lowest pH value, 4.65. Soils 2 and 3 are characterized by a more distinctive decelerated nitrification due to the limiting influence of the pH value of the soils on biological nitrification. In soils 2 and 3, toxic amounts of nitrite appeared (9.1 ppm).[5] These toxic amounts appeared earlier (12th day) in soil 2. This phenomenon is connected with the decrease in the contents of NH_4 and NO_3–N content. Thus, the increased nitrite content is brought into connection with retarded nitrification and denitrification.[19]

The results of the incubation experiments of examined soils with the addition of 100 ppm of NH_4–N are shown in Fig. 2.



(a) (b)

(c)

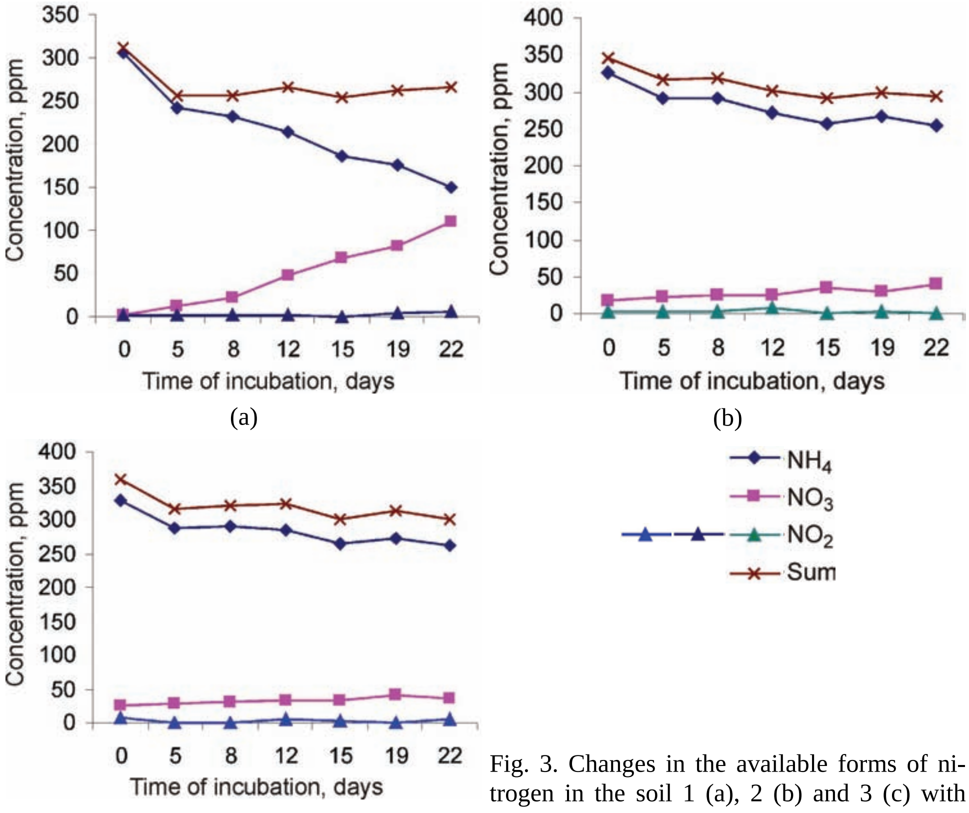
Fig. 2. Changes in the available forms of nitrogen in the soil 1 (a), 2 (b) and 3 (c) with 100 ppm NH_4–N added.

Regardless of the applied $(NH_4)_2SO_4$, there was no increased nitrogen mineralization in the soils in relation to the treatment without addition of this salt (soil+water).

The fastest changes in the processes of nitrification and denitrification were registered in soil 1. This is understandable taking into consideration the higher pH value of this soil and the fact that in the soil a constant source of NH_4–N was present, being the result of the $(NH_4)_2SO_4$ addition. This soil contained the highest amounts of NO_2–N (35.7 ppm), which is the consequence of intense processes of nitrification and denitrification, as shown in a previous paper.[16]

In soils 2 and 3 that had a lower pH value, *i.e.*, they were more acid, the NH_4–N quantities decreased slower due to retarded biological nitrification, decreasing in that way the denitrification. In addition to acidity, these two processes were decelerated due to the influence of the addition of $(NH_4)_2SO_4$ (100 ppm).[20] In these soils (2 and 3), smaller quantities of NO_2–N were observed compared to soil 1. However, in the later phases of incubation (on the 15th day, soil 2 – 14.0 ppm, soil 3 – 18.9 ppm) these quantities were also toxic for plants and microorganisms (>5 ppm).[5] The presence of toxic amounts of NO_2–N can be explained as resulting from processes of chemical nitrification[16] and chemodenitrification.[21] This was confirmed by the decreased total content of mineral forms of nitrogen at the end of the experiment.

The results of the incubation experiments of the examined soils with 300 ppm NH_4–N added are shown in Fig. 3.



(a)

(b)

(c)

Fig. 3. Changes in the available forms of nitrogen in the soil 1 (a), 2 (b) and 3 (c) with 300 ppm NH_4–N added.

When larger quantity of NH_4–N (300 ppm) was added, besides the influence of the low pH value of soil 1, an inhibition effect of NH_4–N on the development of biological nitrification and denitrification was also present, so that the deceleration of these processes was greater.

The process of biological nitrification still occurred in soil 1, but more slowly than in the experiment when a smaller quantity of NH_4–N was added. Under these conditions, nitrites were formed but in smaller quantities and the toxic amount appeared only at the end of the incubation experiment (on the 22nd day – 6.3 ppm).[5]

In more acid soils (2 and 3), the process of biological nitrification nearly ceased and nitrites did not appear in larger quantities. In both soils, the toxic amount of 6.3 ppm appeared in the middle of incubation period (12th day). However, the content of NH_4–N decreased during the incubation period, although slowly, as well as the quantity of mineral forms of nitrogen. This indicates the occurrence of gaseous losses, mostly chemodenitrification. Hence, nitrites were not accumulated. This was confirmed in experiments when $NaNO_2$ was added.[16]

CONCLUSIONS

On the basis of the obtained results, it is possible to draw the following conclusions:

1. In the examined soils, the low pH value of the soils caused retardation of biological nitrification and denitrification, which was the reason for the occurrence of nitrite nitrogen in toxic quantities.

2. The other source of increased and toxic amounts of nitrite nitrogen in the examined soils (soils of higher acidity) was chemodenitrification upon application of $(NH_4)_2SO_4$ (100 and 300 ppm NH_4–N), when biological nitrification and denitrification was considerably retarded and even ceased.

3. Nitrites formed in the chemodenitrification process underwent spontaneous oxidation to nitrates by a chemical process (chemical nitrification).

4. On the basis of obtained results, the application of lower doses of nitrogen fertilizers in fields on pseudogley soil is recommended (50 ppm) in relation to the doses applied on neutral or alkaline soils. Using the lower doses, nitrogen losses due to denitrification might be decreased. The possibility of the occurrence of toxic quantities of nitrite, which often damage or destroy plants, could also be decreased.

ИЗВОД

СПЕЦИФИЧНОСТИ ТРАНСФОРМАЦИЈЕ МИНЕРАЛНИХ ОБЛИКА АЗОТА У КИСЕЛИМ ЗЕМЉИШТИМА

МИРЈАНА КРЕСОВИЋ[1],МИОДРАГ ЈАКОВЉЕВИЋ[1],СРЂАН БЛАГОЈЕВИЋ[1] и СРБОЉУБ МАКСИМОВИЋ[2]

[1]Пољопривредни факултет, Немањина 6, 11080 Земун и [2]Институт за земљиште,
Теодора Драјзера 7, 11000 Београд

Истраживања су обављена на земљишту типа псеудоглеј из околине Шапца. Испитивана земљишта спадају у групу киселих и јако киселих земљишта. Промене минералних облика азота су праћене помоћу краткотрајних инкубационих огледа (влажност 30 % и температура 20 °C), са и без додатка 100 и 300 ppm NH_4–N. Током инкубације утврђене су

повишене и токсичне количине нитрита, које су се у земљишном раствору задржавале више дана и недеља. Утврђене количине нитрита су се јављале као последица успорене биолошке нитрификације (код мање киселог земљишта), док су при додатку 100 и 300 ppm NH_4–N оне настајале у процесу хемоденитрификације. У испитиваним земљиштима се одвијала и спонтана оксидација насталих нитрита хемијским путем (хемијска нитрификација). Да би се смањили губици азота процесом денитрификације и стварање токсичних количина нитрита на псеудоглејном земљишту се може препоручити примена нижих доза азотних ђубрива у односу на дозе које се примењују код неутралних и алкалних земљишта.

(Примљено 21. марта, ревидирано 30. јуна 2008)

REFERENCES

1. R. J. Haynes, in *Mineral Nitrogen in the Plant-Soil System*, T. T. Kozlowski, Ed., Wisconsin, Madison, 1986, p. 52
2. S. U. Sarathchandra, *Plant Soil* **50** (1978) 99
3. L. G. Morrill, J. E. Dawson, *Soil Sci. Soc. Am. Proc.* **31** (1967) 757
4. L. C. Burns, R. J. Stevens, R. J. Laughline, *Soil Biol. Biochem.* **28** (1996) 609
5. C. A. Black, *Soil–Plant Relationships*, 2nd Ed., Wiley, London, 1968, p. 464
6. R. J. Bartlett, *Soil Sci. Soc. Am. J.* **45** (1981) 1054
7. J. O. Reuss, R. L. Smith, *Soil Sci. Soc. Am. Proc.* **29** (1965) 267
8. M. P. Korsakova, *Microbiology* **2** (1941) 3
9. J. R. Haynes, R. R. Sherlock, in *Mineral Nitrogen in the Plant–Soil System*, T. T. Kozlowski, Ed., Wisconsin, Madison, 1986, p. 242
10. K. L. Sahrawat, *Plant Soil* **65** (1982) 281
11. L. C. Burns, R. J. Stevens, R. V. Smith, J. E. Cooper, *Soil Biol. Biochem.* **27** (1995) 47
12. M. N. Court, R. C. Stephens, J. S. Wais, *Nature* **194** (1962) 1263
13. W. M. Lewis, D. P. Morris, *Trans. Am. Fish. Soc.* **115** (1986) 183
14. F. B. Eddy, E. M. Williams, in *Water Quality for Freshwater Fish*, G. Howells, Ed., Gordon and Breach Science Publishers, Yverdon, 1994, p. 117
15. *Soil Map of Serbia* (scale 1:50.000), The Institute for Soil Science, Belgrade, 1964
16. M. Jakovljević, M. Kresović, S. Blagojević, S. Antić-Mladenović, *J. Serb. Chem. Soc.* **70** (2005) 765
17. J. M. Bremner, in *Methods of Soil Analysis*, C. A. Black, Ed., Madison, Wisconsin, 1965, p. 1324
18. R. J. Muluaney, in *Methods of Soil Analysis*, J. M. Bigham, Ed., Madison, Wisconsin, 1996, p. 1123
19. Q. R. Shen, W. Raw, Z. H. Cao, *Chemosphere* **50** (2003) 747
20. S. S. Malhi, W. B. McGill, *Soil Biol. Biochem.* **14** (1982) 393
21. C. J. Smith, P. M. Chalk, *Soil Sci. Soc. Am. J.* **44** (1980) 277.