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NITROGEN TRANSFORMATION IN ACID SOILS SUBJECTED TO PH VALUE CHANGES

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Abstract – The aim of this investigation was to determine which application of fertilizer and lime material does not form toxic quantities of nitrite nitrogen and when the losses by denitrification are the lowest in the examined acid soils. Investigations were performed on pseudogley soils of different acidity. Changes of available nitrogen forms were examined by the method of short-term incubation experiments. Experimental treatments were without the use of mineral fertilizers and with application of $(NH_4)_2SO_4$ (100 ppm of NO_3-N) and KNO_3 (100 ppm of NO_3-N); with and without addition of $Ca(OH)_2$ (50% of full neutralization and full neutralization). When $(NH_4)_2SO_4$ was used, nitrites occurred in both examined soils as a result of decelerated nitrification and when KNO_3 was added as a result of chemical denitrification. Application of $Ca(OH)_2$ caused the intensification of mineralization, nitrification and biological denitrification processes. When a higher dose of lime material was used (full neutralization), nitrites occurred in larger quantities as a result of the strengthening of nitrification and denitrification processes. Application of a lower lime dose caused nitrite occurrence in smaller quantities. Therefore, in these soils as well as in soils of similar chemical properties, application of lower doses of lime material can be recommended (<50% of full neutralization) as well as the application of ammonium fertilizer, bearing in mind that in such conditions losses of added fertilizer in the denitrification process are reduced and the occurrence of nitrites as an intermediate product of this process is prevented.

Key words: Nitrification, mineralization, denitrification, lime, nitrite

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INTRODUCTION

Nitrogen transformation in acid soils is significantly different from neutral and alkaline soils. It is well known that chemoautotrophic nitrification is a strictly specific process in which microorganisms from the group of real bacteria act (*Nitrosomonas, Nitrosococcus, Nitrosospira, Nitrosobus, Nitrosovibrio and Nitrobacter)* (Paul and Clark, 1996; De Boer and Kowalchuk, 2001). Nitrification bacteria are sensitive to the effect of environment where soil pH value represents one of the limiting factors (Haynes, 1986). Therefore, the pH value of 4.5 (water) is considered as a lower threshold for the activity of autotrophic nitrificators (Sahrawat, 1982; Paul and Clark, 1996). In acid soils, due to a higher sensitivity of nitrate bacteria to a low soil pH value, nitrification is often significantly decelerated with the consequence of nitrite accumulation (Black, 1968). Under these conditions, oxidation speed of NH_4-N is higher than the oxidation of NO_2-N (Smith et al. 1997) and thus nitrites accumulate in the soil. The formed nitrites in acid soils, even in small quantities (a few ppm) can be toxic to plants and microorganisms (Black, 1968; Shen et al. 2003). A high level of ammonium nitrogen in soil can also inhibit Nitrobacter activity, with the consequence of nitrite accumulation in the soil (Morrill and Dawson, 1967; Malhi and McGill, 1982; Shen et al. 2003; Burns et al. 1996). In acid soils, a toxic level of aluminum (more than 5 mg/100 g) can significantly reduce nitrification activity (Brar and Giddens, 1968; Jakovljević et al. 2005). Liming is applied in acid soils to prevent negative influences of too low pH values. Besides this, application of lime to acid soils stimulates nitrification, often more intensively than ammonification (Nyborg and Hoyt, 1978). However, the increase of pH value, besides intensification of the nitrification process, creates favorable conditions for strengthening the process of biological denitrification, where nitrites occur as an intermediate product and losses of gaseous nitrogen from added mineral fertilizers increase (Firestone, 1982).

The aim of these investigations was to establish which application of fertilizer and lime material doesn't form toxic quantities of nitrite nitrogen and when the losses by denitrification are the lowest in the examined acid soils.

MATERIAL AND METHODS

Investigations were performed on pseudogley soil from two different regions (eastern Serbia – soil 1 and western Serbia – soil 2). Soil samples were taken from a depth of 0-20 cm, air-dried, triturated and sifted with a 2 mm sieve. The basic chemical properties of the examined soils were determined and the incubation experiment was performed.

The basic chemical properties of the investigated soils were determined by the following methods:

- pH value was determined potentiometrically with a combined glass electrode, by mixing soil with water and 1M KCl solution (ratio 1:2.5).

- Organic carbon and humus were determined by the dichromate method (Mineev, 2001).

- Available phosphorous and potassium were determined by the Al method.

- Content of exchangeable aluminium was determined by the titration method (Sims, 1996).

- Hydrolytic acidity was established by Kappen's method (Mineev, 2001).

- Dosage of $Ca(OH)_2$ was calculated in accordance to hydrolytic acidity (Mineev, 2001).

Incubation experiment

Changes of available nitrogen forms in the investigated soils were monitored by the method of short-term incubation experiments in accordance to a well-known procedure (Bremner, 1965) with treatments as follows: soil+water; soil+100 ppm of NH_4-N and soil+100 ppm of NO_3-N. Changes of pH values were made by adding $Ca(OH)_2$ with the following treatments: neutralization of 50% of hydrolytic acidity + 100 ppm of NH_4-N, i.e. 100 ppm NO_3-N and full neutralization + 100 ppm of NH_4-N, i.e. 100 ppm of NO_3-N.

Experiments were performed with soil moisture of 30% and temperature of 30°C. The incubation experiment was performed as follows: 10 g of soil with the addition of 3 ml of water was placed in glass jars of 150 ml volume for the first variant of the experiment, i.e. 3 ml of ammonium sulfate solution with 100 ppm of NH_4-N and 3 ml of potassium nitrate solution with 100 ppm of NO_3-N for the second and the third variant.

Lime doses used for neutralization were: 11.4 and 22.8 mg of $Ca(OH)_2$ per 10 g of soil for soil 1 and 5.9 and 11.8 mg for soil 2.

The glass jars were closed with a thin plastic foil and the incubation was performed at a temperature of 30°C. When the anticipated incubation period elapsed (2, 4, 6 and 8 days) the jars were opened and 100 ml of 2M KCl solution was added. The jars were shaken for one hour and then filtered. In the obtained filtrate, the content of available forms of nitrogen (NH_4-N and NO_3-N) was determined by the distillation method (Bremner, 1965) and NO_2-N was determined by the colorimetric method (Puttanna and Prakas Rao, 1986).

RESULTS AND DISCUSSION

Chemical properties of examined soils are presented in Table 1.

Soil 1 had a distinctive acid reaction (pH in 1M KCl – 3.70), and an increased content of exchangeable aluminium and high hydrolytic acidity. Thus, this soil had unfavorable conditions, for the development of chemoautotrophic nitrification and biological denitrification processes and favorable conditions for the development of chemodenitrification.

Table 1. Basic chemical properties of examined soils

Soils	pH		Humus (%)	Total N (%)	C/N	Available (mg/100g)		Exchangeable Al (mg/100g)	Hydrolytic acidity (m.ekv./100g)
	Water	M KCl				P_2O_5	K_2O		
Soil 1	4.80	3.70	2.08	0.10	12.0	6.2	16.2	5.9	6.0
Soil 2	5,50	4.35	1.82	0.09	10.4	13.2	15.0	0.4	3.1

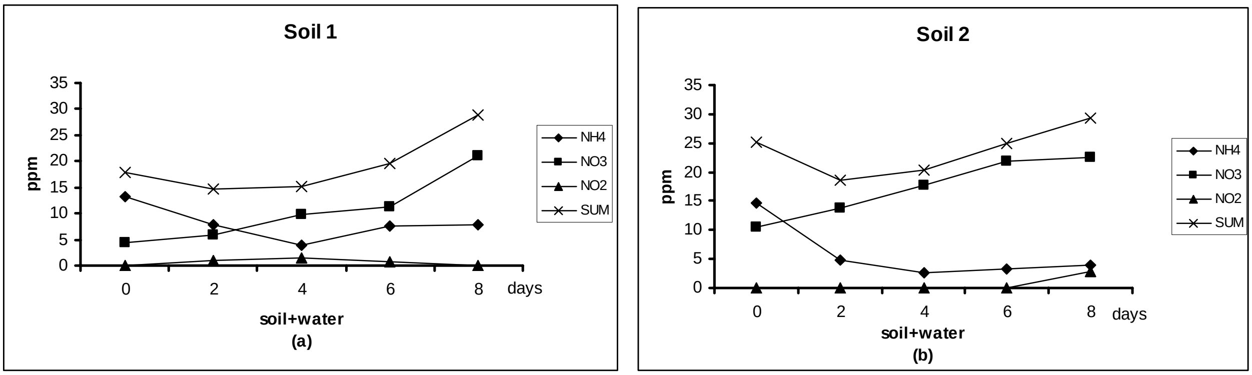

Figure 1. Changes of available nitrogen forms in examined soils.

Soil 2 had a higher pH value (4.35 in 1M KCl), although both soils belong to a category of very acid soils. This soil had a much lower content of exchangeable aluminium and lower humus content but a higher content of available phosphorous (Munever and Wallum, 1977). Due to the lower humus and total nitrogen content, this soil had more unfavorable conditions for mineralization of soil nitrogen.

Figure 1 (a and b) presents the results of the incubation experiment for the examined soils with addition of water only.

The initial content of available nitrogen in soil 1 was lower than in soil 2. This particularly refers to the content of NO_3-N (4.5 ppm). In this soil, the process of chemoautotrophic nitrification was slowed down. This can be observed from the occurrence of NO_2-N on the 4th day of the incubation period (1.47 ppm). After that, NO_2-N disappeared in the chemodenitrification process (Haynes and Sherlock, 1986; Paul and Clark, 1996). From the 6th to 8th day of the incubation period, the process of nitrification was intensified. Taking into consideration the conditions in this soil, the formed nitrates are connected to the process of chemical nitrification (Bartlett, 1981; Allison,1973). After the initial decrease of NH_4-N, from the 4th day of incubation, its content increased, indicating the process of mineralization.

In soil 2, that had a lower acidity (Fig. 1b), the process of nitrification developed much faster from the very beginning of the incubation period when the NH_4-N content decreased. By the end of the incubation period, nitrification was slower due to the occurrence of chemodenitrification. This can be observed by the occurrence of formed nitrites at the end of the incubation period (2.94 ppm).

Figure 2 (a, b and c) shows the results of the incubation experiment for the soil 1, with the

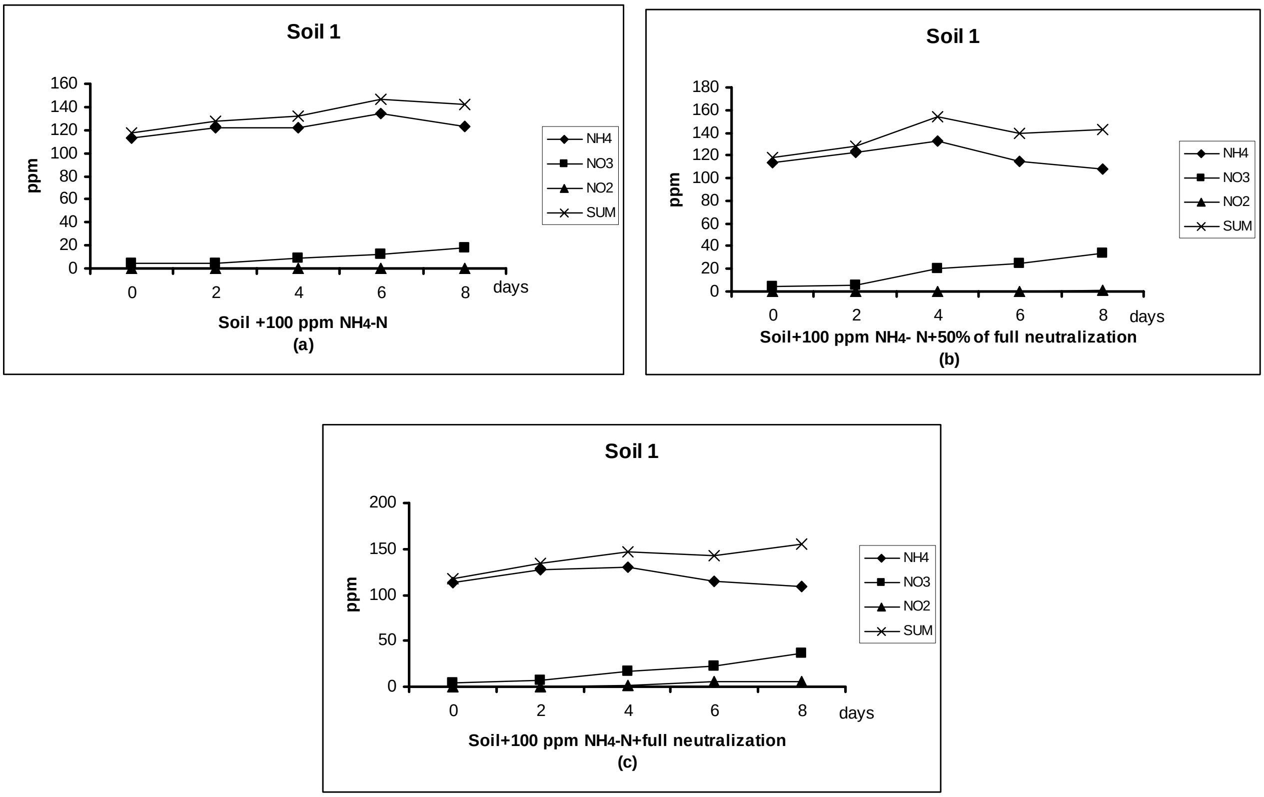

Figure 2. Changes of available nitrogen forms in soil 1 with application 100 ppm of NH_4-N and $Ca(OH)_2$.

addition of $(NH_4)_2SO_4$ (100 ppm of NH_4-N), with and without applied doses of $Ca(OH)_2$.

When only $(NH_4)_2SO_4$ was added (Fig. 2a), the process of nitrification was very retarded so the quantities of formed NO_3-N were lower than in the case of the addition of water only (Fig. 1a). The increase in the quantity of formed NO_3-N could not be observed before the 4th day of incubation. The additional deceleration of the process of nitrification was certainly a consequence of $(NH_4)_2SO_4$ application that made the soil more acid and reduced nitrification. Nitrites appeared in small quantities during the whole incubation period (0.1 – 0.3 ppm). The quantities of ammonium nitrogen increased from the 2nd to the 6th day, indicating the development of the mineralization process. The low pH value of the soil had much less influence on mineralization than on nitrification (Rorison, incubation experiment for the soil 1 when KNO_3 was used, without and with the application of $Ca(OH)_2$.

When KNO_3 as a neutral salt was added (Fig. 3a), there was an increase of NH_4-N content, i.e. the process of mineralization was more intense. The content of NO_3-N decreased slowly in the first half of the incubation period; there was an insignificant increase in the second half and nitrites did not appear.

The effect of a lower lime dose usage (Fig. 3b) on the process of available nitrogen transformation in examined soil 1 was small. However, nitrites appeared on the 4th day (2.3 ppm) and on the 8th day of the incubation period (0.8 ppm).

The curves had a similar trend when a higher dose of $Ca(OH)_2$ was applied. However, larger

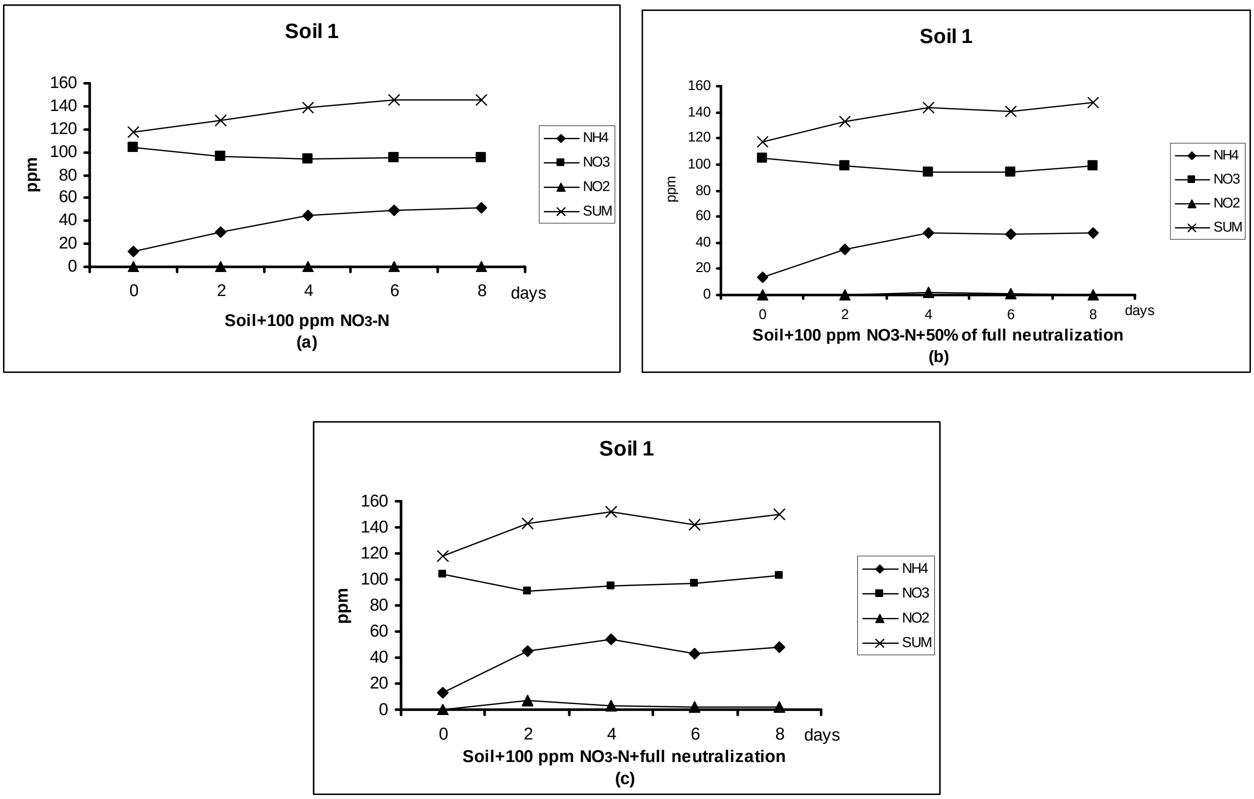

Figure 3. Changes of available nitrogen forms in soil 1 when 100 ppm of NO_3-N and $Ca(OH)_2$ were used.

quantities of nitrites occurred (7.4; 3.2, 2.5 and 1.7 ppm) as a result of increased biological nitrification and denitrification processes.

Figure 4 (a, b and c) indicates the results of the incubation experiment for the soil 2 when $(NH_4)_2SO_4$ was applied, without and with addition of $Ca(OH)_2$.

When $(NH_4)_2SO_4$ was applied to the soil 2 that is less acid and poorly supplied with humus, a decrease of NH_4-N was noticed. This was a consequence of intensification of the chemoautotrophic nitrification process. It could be assumed that the decrease of NH_4-N content could also have occurred due to ammonium nitrogen fixation in the soil. Nitrites appeared in traces (0.1 – 0.3 ppm), as was the case in soil 1. In contrast to soil 1, when $(NH_4)_2SO_4$ was added to soil 2, larger quantities of NO_3-N were formed; this was a consequence of the the available nitrogen transformation between applied treatments.

A larger difference appeared only in treatment with full neutralization (Fig. 5c) where nitrification and mineralization processes were more intensive. Nitrites appeared in quantities of 1.2 – 4.0 ppm.

On the basis of the presented results we concluded that the chemoautotrophic nitrification in soil 1 was significantly decelerated, due to its lower pH value and increased content of available aluminium in relation to soil 2. The consequence of this was the appearance of nitrites (Kresović et al. 2009). The process of mineralization was more intensive in soil 1 than in soil 2 due to a higher humus content in this soil, as shown by Charoulis et al. 2005. When 100 ppm of NH_4-N was applied, nitrites appeared in both soils at the end of the incubation period due to retarded nitrification,

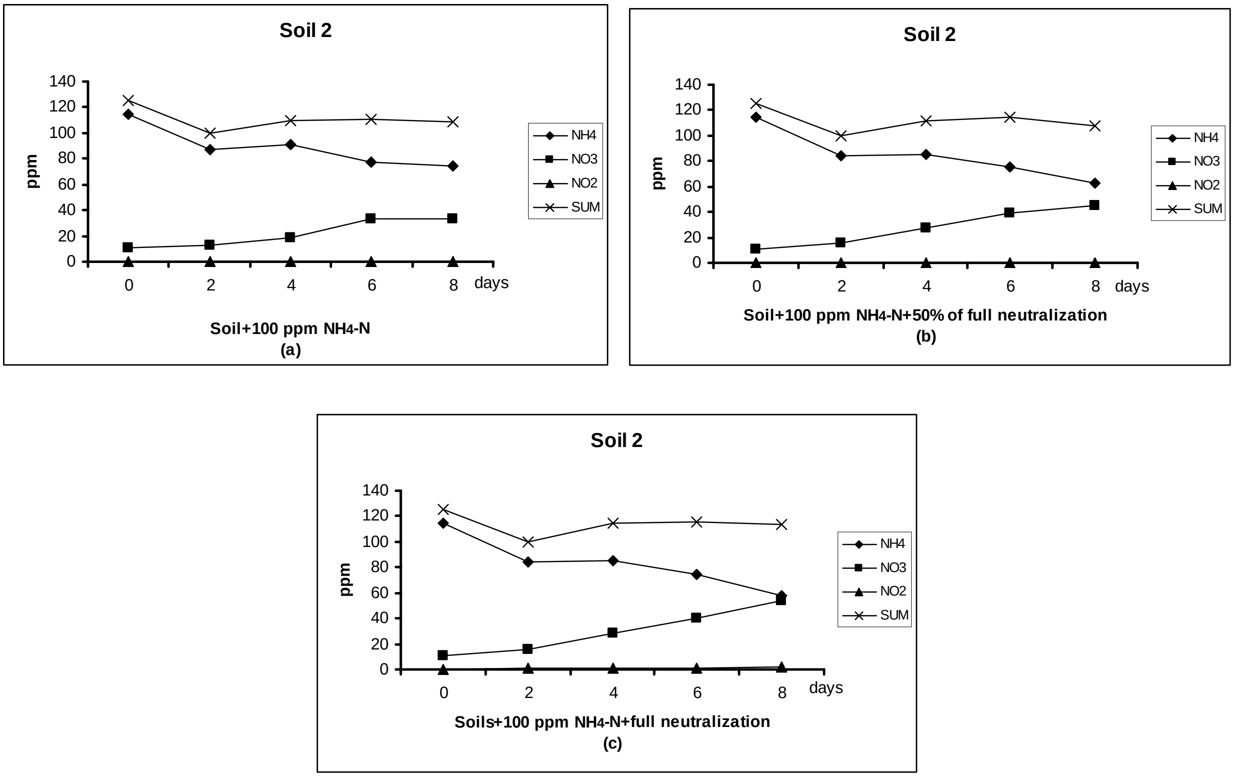

Figure 4. Changes of available nitrogen forms in soil 2 when 100 ppm of NH_4-N and $Ca(OH)_2$ were applied.

whereas the application of 100 ppm of NO_3-N caused higher amount of nitrites at the beginning (soil 2) and in the middle of incubation period (soil 1) due to the development of biological and chemical denitrification. Application of $Ca(OH)_2$, i.e. increasing the soil pH value, caused intensification of the mineralization, nitrification and denitrification processes. When a higher dose of lime material was applied, nitrites appeared in larger quantities in soil 1 (0.4 – 7.4 ppm) and in soil 2 (0.8 – 4.0 ppm) as a result of strengthening the simultaneous biological processes of nitrification and denitrification. The quantities of formed nitrites in both examined soils were lower in treatments with lower doses of lime material.

In these and in soils of similar chemical properties, the application of lower doses of lime material can be recommended (less than 50% from full neutralization), as well as the application of ammonium fertilizers. In these conditions, the losses of added fertilizer in the denitrification process are reduced and the occurrence of nitrites as a by-product of this process can be prevented.

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ТРАНСФОРМАЦИЈА АЗОТА У КИСЕЛИМ ЗЕМЉИШТИМА ПРИ ПРОМЕНИ pH ВРЕДНОСТИ

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Истраживања су обављена на псеудоглејном земљишту различите киселости. Циљ ових истраживања је био да се утврди при којој примени ђубрива и кречног материјала се не образују токсичне количине нитрита и када су губици азота денитрификацијом најмањи. Применом амонијум сулфата, код оба испитивана земљишта, образовали су се нитрити као последица успорене нитрификације а при додатку калијум нитрата нитрити су настајали услед биолошке и хемијске денитрификације. При примени виших доза $Ca(OH)_2$ (пуна неутрализација) нитрити су се јављали у већим количинама као резултат јачања процеса нитрификације и денитрификације, док је при нижим дозама кречног материјала количина образованих нитрита била мања. Ради спречавања појаве нитрита и смањења губитака азота процесом денитрификације, код оваквих и земљишта сличних хемијских својстава, може се препоручити примена амонијачних ђубрива и употреба нижих доза кречног материјала.