Article

Examination of nitrification inhibition by sorghum (*Sorghum bicolor*) in soil around its roots

Adel Ghoneim[1,2], **Abdulla Al-Modaihsh**[1], **Saied Naeem**[2], **Tamer Metwally**[2], **Elsaied Gewailly**[2], **Azza Ebid**[3]

[1]Soil Science Department, College of Food and Agricultural Sciences, King Saud University, P.O. Box 2460, Riyadh, 11451, Saudi Arabia

[2]Agricultural Research Center, Rice Research and Training Center (RRTC), Sakha, 33717, Kafr El-Sheikh, Egypt

[3]Department of Biology, College of Science, Princess Nora Bint Abdul Rahman University, P. O. Box 376114 Riyadh 11335, Saudi Arabia

E-mail: aghoneim@ksu.edu.sa

Received 2 September 2013; Accepted 18 October 2013; Published online 1 March 2014

Abstract

Biological nitrification inhibition refers to release some chemical substances from plant root that suppresses/slowdown soil nitrification. This study was conducted to clarify whether sorghum (*Sorghum bicolor*) inhibit nitrification in soil around the root. Sorghum cultivated in pots filled with a brown lowland soil and examined nitrification rate in the soil around its root comparing with bare soil. Two experiments were conducted. In the first experiment, sorghum was cultivated in a growth chamber and soil sample were collected four times during the growth period. Nitrogen in the form of $(NH_4)_2SO_4$ (120 mg N kg^{-1} soil) was mixed with the soil samples and incubated at 30°C, for 21 days. Nitrification rates were estimated based on NO_2^-+NO_3^--N accumulation per unit time. Results showed that in sometime nitrification rate in the soil around sorghum root was lower than that in the bare soil however, in other times there were no difference between them. Second experiment was conducted by using soil samples collected from the pots in which sorghum was cultivated in a greenhouse. The results showed nitrification in the soil around sorghum root was lower than that in the bare soil. Nitrification was inhibited in soil around the sorghum roots, however, this inhibition varied with incubation period. The differences of N application showed a little effect of nitrification inhibition rates.

Keywords sorghum; rhizosphere soil; nitrification inhibition.

Proceedings of the International Academy of Ecology and Environmental Sciences
ISSN 2220-8860
URL: http://www.iaees.org/publications/journals/piaees/online-version.asp
RSS: http://www.iaees.org/publications/journals/piaees/rss.xml
E-mail: piaees@iaees.org
Editor-in-Chief: WenJun Zhang
Publisher: International Academy of Ecology and Environmental Sciences

1 Introduction

Nitrification is oxidation of ammonium (NH_4^+) to nitrate (NO_3^-) by specific soil microorganisms. It changes relatively immobile NH_4^+-N to highly mobile NO_3^--N, which can result in nitrogen losses from nitrification-

denitrification and NO_3^- leaching. Problems of low N use efficiency due to N losses through nitrification-denitrification, leaching constantly being reported (Glass, 2003; Giles, 2005). About 70% of 100Tg of N applied as N fertilizer is lost through nitrification and other transformation, which translates to about 17 billon US dollars, in addition the unknown costs from environmental impact such as NO_3^- pollution and eutrophication of surface water and gas emission (Apr et al., 2002; Giles, 2005). Considerable effort has been invested in the identification of chemicals that can inhibit, or at least slow the nitrification process (Slangen and Kerkhoff, 1984; Prasad and Power, 1995; McCarty, 1999). Some plants have been reported to have the ability to release some biological active substances from their roots that suppresses nitrification. These compounds are called as biological nitrification inhibition (BNI). Biological nitrification inhibition refers to the release of root exudates capable of inhibiting bacteria responsible for the nitrification process. Subbarao et al. (2006a), Subbarao et al. (2006b), Subbarao et al. (2007a) and Subbarao et al. (2007b) showed import new findings of the role of plants in moderating nitrification in soil. However, it was not fully clarified whether these plants which showed strong nitrification inhibition in the assay really inhibit nitrification in soil where the plants grow. Sorghum (*Sorghum bicolor*) had been reported to possess inherent ability to release some biochemical compounds from their roots that could inhibit nitrification in soils, a phenomenon known as biological nitrification inhibition (Subbarao et al., 2007a; Hossain et al., 2008). The study was conducted to clarify evidence of the existence of biological nitrification inhibition function and whether sorghum inhibit nitrification in soil around its root.

2 Materials and Methods

2.1 Soil used for the experiment

Fine-textured brown lowland soil (Haplic Brown Lowland Soils, USDA Soil Taxonomy) was collected from Crops and Field Laboratory, Agriculture and Forestry Research Center, Saitama Prefecture, Japan between 0-30cm depth, sieved with 2.0 mm sieve, mixed thoroughly and kept in plastic bags in a greenhouse. The soil properties were as follows: pH (H_2O) 6.33, total N 0.69 g kg^{-1} dry soil, total C 6.0 g kg^{-1} dry soil, C/N ratio 8.7, NH_4^+-N 0.98 mg $kg^{-1}$and NO_3^--N 0.80 mg kg^{-1}, clay 20%, silt 33% and sand 47%.

2.2 Treatments

2.2.1 Growth chamber experiment

Seeds of sorghum (hybrid sorgo) was germinated at 25°C in trays containing sand and watered with distilled water. Eight seedlings, one-week old were sown in each pot (0.0884 m^2), and the seedlings were thinned to four per pot 7 days after sowing. The pots were prepared with three replications. Four treatments were as follows: (1) soil cultivated with sorghum and N fertilizer was applied @ 50 kg N ha^{-1} as urea one week after planting. (2) Soil cultivated with sorghum without N application. (3) Bare soil with N applied @ 50 kg N ha^{-1} as urea. (4) Bare soil without N application. Plants were grown in a growth chamber with day/night temperature regimes of 25/30°C and 70% relative humidity.

2.2.2 Greenhouse experiment

Seeds of sorghum (PVK-801) were geminated same as in the growth chamber experiment. Four seedlings were sown in each pot (0.0884 m^2), and the plants were thinned to four per pot, one week after sowing. Four treatments were established as follows: 1. Soil cultivated with sorghum without NPK fertilizers (Plant –N). 2. Soil cultivated with sorghum and fertilized with the rate of 100-200-40 Kg NPK ha^{-1}, respectively (Plant +N). 3. Bare soil without sorghum and NPK application (no plant –N) and 4. Bare soil with NPK @100-200-40 Kg NPK ha^{-1} (no plant + N). Phosphorus and potassium applied as basal application before planting in the form of KCl and Na_2HPO_4, respectively while N fertilizer was applied in two splits at planting and one month after sowing. The pots were prepared with 3 replications. Plants were grown in a green house.

2.2.3 Soil incubation to test nitrification inhibition

Represented soil samples were collected from each treatment at 43, 60, 68 and 133 days after sowing in the growth chamber experiment and at 63 and 75 days after sowing in the greenhouse experiment. The soil samples were brought to the laboratory and only the soil adhered to the roots (rhizosphere soil) was removed and used for the following experiment. The soil samples were mixed thoroughly one by one and the initial moisture content was determined. The initial pH (H_2O) of each soil sample was measured at a 1:2.5 soil: water volume ratio with a glass electrode. The soil samples weighed at equivalent weight of 5.0 g dry soil per tube were mixed with 0.6 ml of mixing solution of $(NH_4)_2SO_4$ at the rate of 120 mg N kg^{-1} dry soil, sealed with parafilm and incubated at 30°C. At 0, 3, 7, 14 and 21 days after the incubation, the soil samples were extracted by shaking with 50 ml of $CaSO_4.2H_2O$ for 60 min, and then filtered through Wattman No. 5 filter paper. The filtered solution was then analyzed calorimetrically for NO_2^-+NO_3^--N content (sulfanilamide-α-naphthlamine method) using an Auto Ion analyzer (model AAII, Brant+Luebbe, Germany) (Anonymous, 1974; Litchfield, 1967; Varley, 1966). Accumulated NO_2^-+NO_3^--N was calculated as NO_2^-+ NO_3^--N (at 3, 7, 14 or 21 days after the incubation) - NO_2^- + NO_3^--N (at the beginning of the incubation). Nitrification rates were determined based on nitrite and nitrate accumulation per unit time. Nitrification inhibition rates were calculated using the following equations

Nitrification inhibition rate (%) in the first 7 days:

$$100- \frac{(T2-T1)}{(C2-C1)} * 100$$

where *T2* and *T1* denotes amount of NO_2^-+ NO_3^--N of treatment accumulated at 7 and 0 day of soil incubation, respectively, *C2* and *C1* denotes amount of NO_2^-+ NO_3^--N of control (no plant) accumulated at 7 and 0 day of soil incubation, respectively.

Nitrification inhibition rate (%) from 7^{th} day to 14^{th} day:

$$100- \frac{(T3-T2)}{(C3-C1)} * 100$$

where *T3* and *T2*denotes amount of NO_2^-+ NO_3^--N of treatment accumulated at 14 and 7 day after incubation, respectively, while *C3* and *C1* denotes amount of NO_2^-+ NO_3^--N of control (no plant) accumulated at 14 and 7 day after incubation, respectively.

2.3 Statistical analysis

The experimental data were subjected to analysis of variance using the IRRISTAT statistical package for Windows and the differences among the means were analyzed by the least significant differences (LSD).

3 Results

3.1 Growth chamber experiment

The accumulated amount of NO_2^-+NO_3^--N after 3 days of incubation for the soil samples taken at 43 days after sowing showed that presence of sorghum plant resulted to between 2.87-4.80 mg kg^{-1} of NO_2^-+NO_3^-N accumulation compared with 5.47-18.63 mg kg^{-1} for control (no plant). The changes in NO_2^-+NO_3^--N accumulation increased with increase the incubation time in a similar trend as observed for the first 3 days (Fig. 1 and 2). After 14 days, about 48.93-67.61 mg kg^{-1} of NO_2^- + NO_3^- has been accumulated under plant treatment compared with 71.0-72.03 mg kg^{-1} in bare soil without plant. The NO_2^- + NO_3^--N accumulation was not significantly different (P=0.05) among the treatments for the soil sampled at 43 and 60 days after sowing. In the third and fourth soil sampling at 68 and 133 days after sowing (Fig. 3 and 4) showed significant differences among the treatments. Bare soil without plant showed higher level of NO_2^- + NO_3^--N accumulation than that soil around the sorghum roots during the different incubation time. With increasing the incubation time, the

changes in NO_2^- + NO_3^--N accumulation increased in a similar trend. The lower NO_2^- + NO_3^--N accumulated in the soil sampled from the around of sorghum roots at 68 and 133 days after sowing suggested that nitrification in the soil was less active than that of the bare soil sample (Fig. 3 and 4). Results in Fig. 5 and 6 from the rhizosphere soil incubation test, bare soil showed that higher rates of nitrification than the values obtained from sorghum plot. The rate of nitrification of bare soil was more than six fold greater than the value obtained for the presence of sorghum for the soil sampled at 68 days after sowing (Fig. 5). The rates on nitrification over the incubation period showed a range of 1.6-4.8 mg kg^{-1} day^{-1} of NO_2^- + NO_3^- N, however, the nitrification rates value were higher after 7 days of incubation compared with 14 days after incubation (Fig. 6). Also at 133 days after sowing, soil nitrification rate was higher in bare soil (ranged from 2.45-5.4 mg kg^{-1} day^{-1}) and the lowest in plant without N (1.8-2.7 mg kg^{-1} day^{-1}) as shown in (Fig. 2). There was a narrow change in soil pH between the pre-cropping soil pH (H_2O:6.33) and post-cropping values (Table 1). The bare soil (no-plant) without the N application showed a lower soil pH compared with other treatments. The variation in soil pH values before incubation test across the N application and plant growth were significant. Root exudates of sorghum inhibited soil nitrification by 6-85% in fresh soil at 7-14 days after incubation (Fig. 7). Its seems that the biological nitrification inhibition activity from sorghum root exudates once released, is stable in inhibiting soil nitrification up to two weeks.

Table 1 pH (H_2O) values (mean ± standard deviation) of soil used for the incubation experiment.

	Days after sowing			
Treatments	43day	60day	68day	133day
Plant – N	6.54b ± 0.05	6.44a ± 0.04	6.42a ± 0.01	6.52a ± 0.04
Plant + N	6.44c ± 0.05	6.52a ± 0.01	6.35b ± 0.01	6.48ab ± 0.14
Bare soil – N	6.51bc ± 0.03	6.38a ± 0.01	6.41a ± 0.01	6.36b ± 0.07
Bare soil + N	6.76a ± 0.06	6.52a ± 0.62	5.85c ± 0.04	5.71c ± 0.19

Plant–N; cultivated sorghum without N application, Plant+N; cultivated sorghum with N application, Bare soil – N; no sorghum without N application, Bare soil +N; no sorghum with N application; Different letters in each column denote significant differences (LSD, P<0.05, n=3).

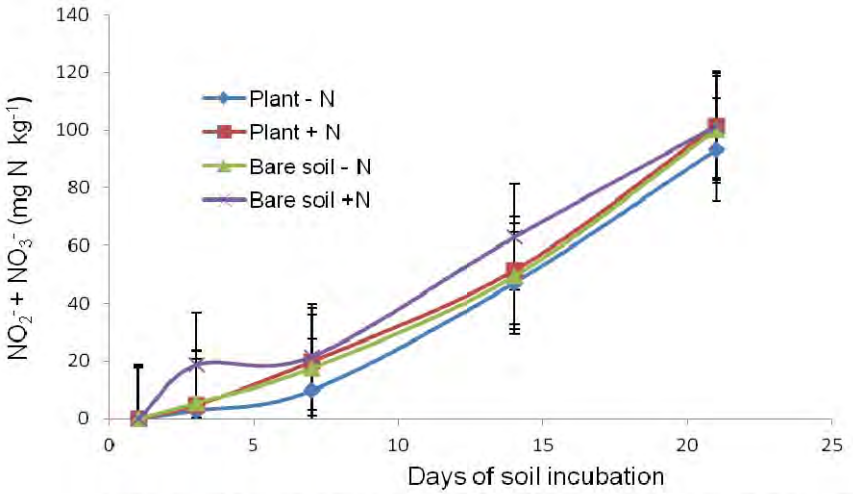

Fig. 1: Amount of NO_2^- + NO_3^- -N released over incubation time in soil at 43 days after sowing. Vertical bars represent ± standard error (SE, n=3).

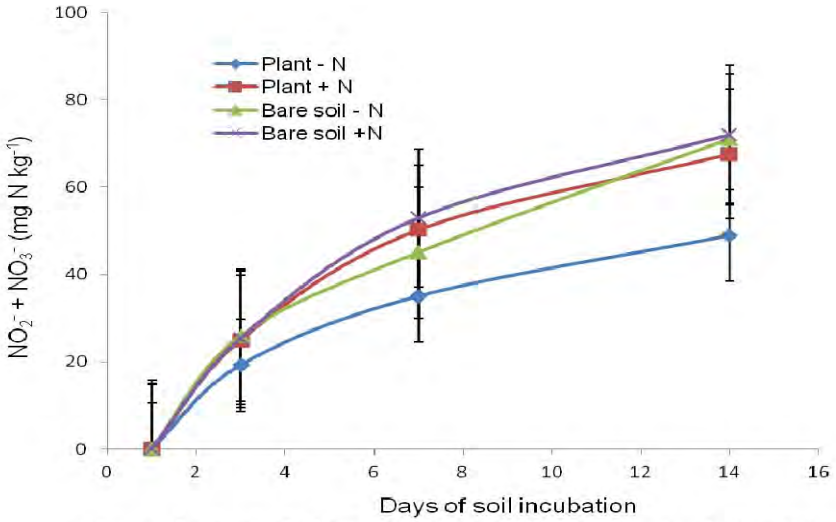

Fig. 2: Amount of NO_2- + NO_3^- -N released over incubation time in soil at 60 days after sowing. Vertical bars represent ± standard error (SE, *n*=3).

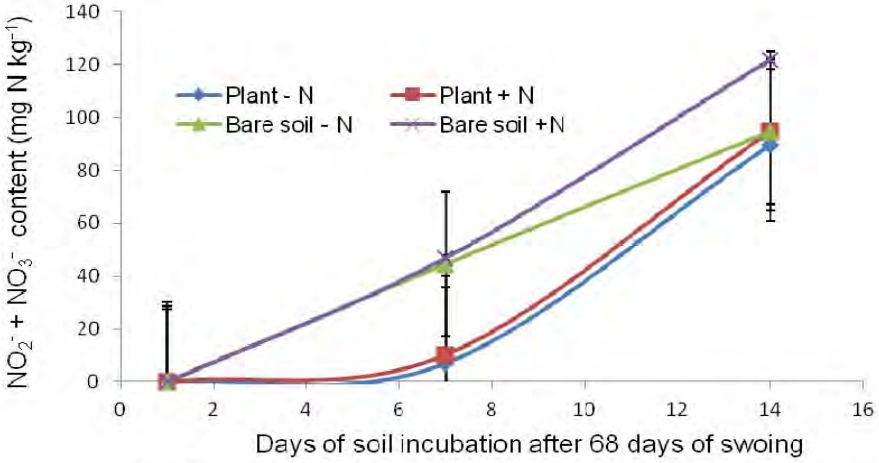

Fig. 3: Differences among treatments in NO_2^- + NO_3^- -N acculmulated in rhizospher soil during the incubation. Vertical bars represent ± standard error (SE, *n*=3).

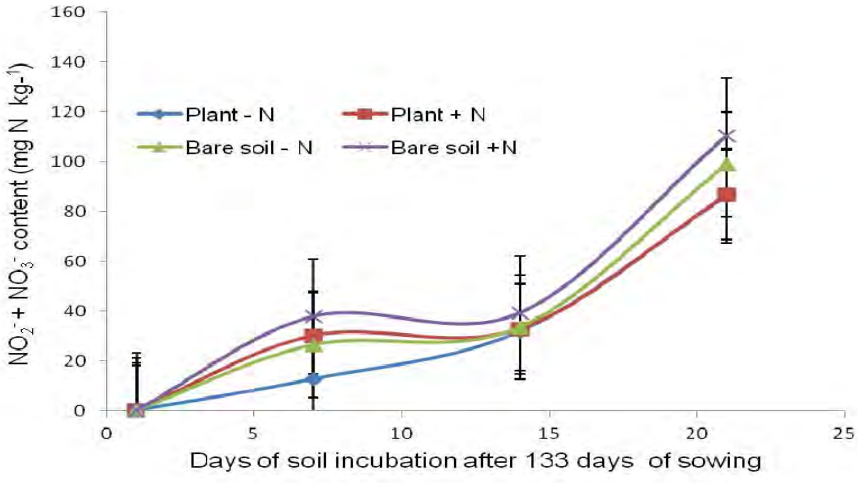

Fig. 4: Differences among treatments in NO_2^- + NO_3^- -N accumulation in rhizosphere soil during incubation. Vertical bars represent ± standard error (SE, *n*=3).

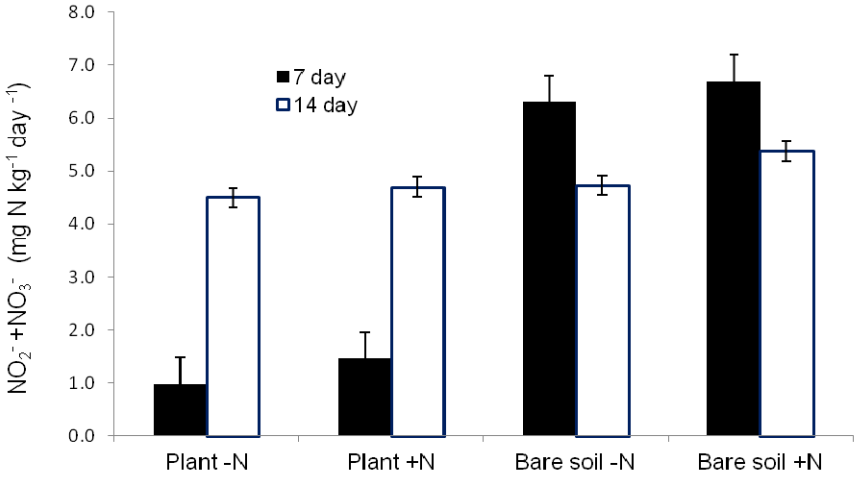

Fig . 5: Differences in nitrification rates from incubated soil during 7 and 14 days (68 DAS). Vertical bars represent ± standard error (SE, *n*=3)

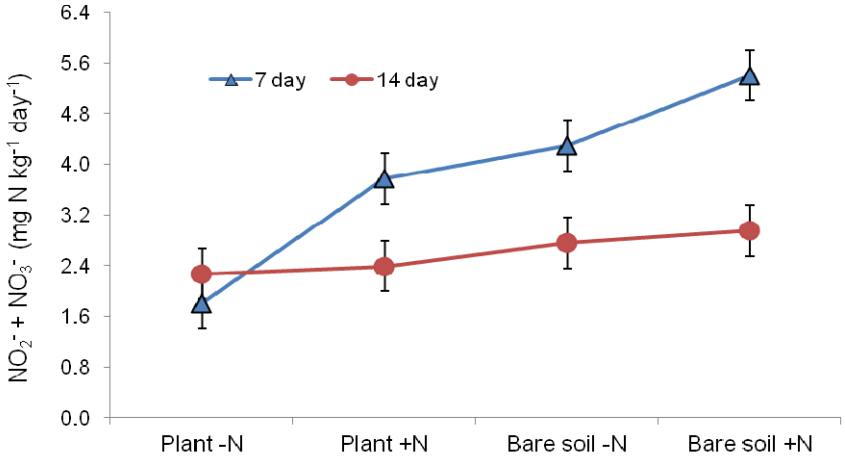

Fig. 6: Differences in nitrification rates from incubated soil during 7 and 14 days (133 DAS). Vertical bars represent ± standard error (SE, *n*=3).

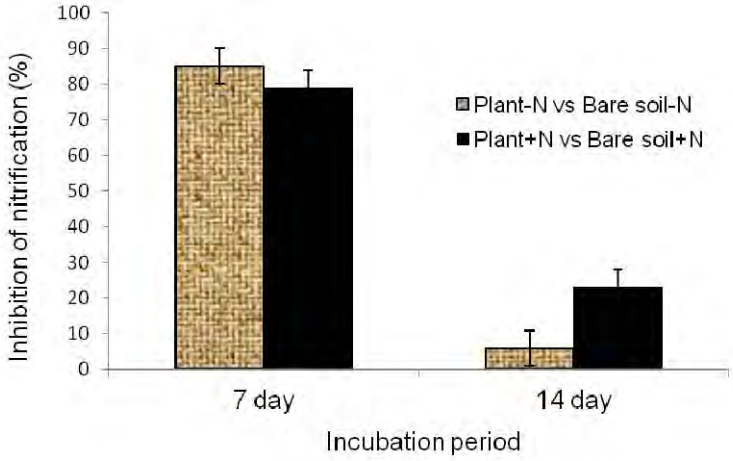

Fig. 7: Inhibition of nitrification by soil in which sorghum had been grown and barren soil. Vertical bars denote ± standard error (SE, *n*=3).

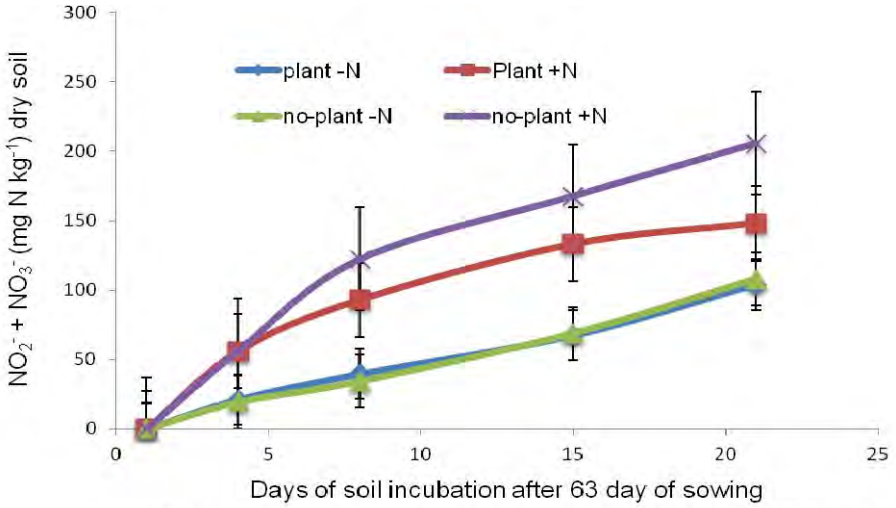

Fig. 8: Differences among treatments in nitrite + nirate formation in rhizospher soil during the incubation. Vertical bars represent ± standard error (SE. *n*=3).

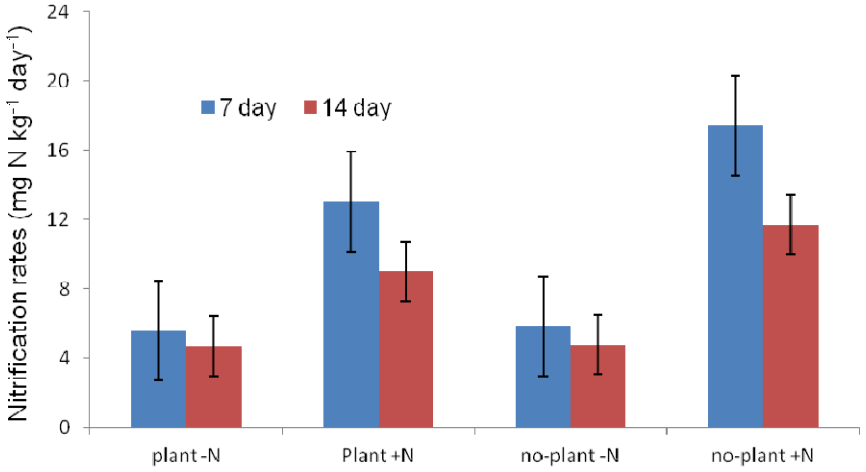

Fig. 9: Differnces in nitrification rates from soil incubation test (63 DAS-greenhouse experiment. Vertical bars represent ± standard error (SE, *n*=3).

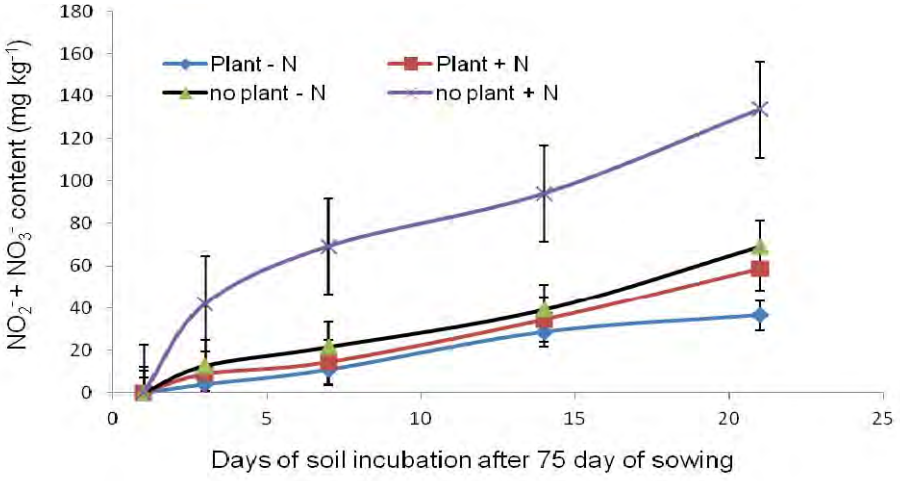

Fig. 10: Amount of NO_2^- +NO_3^- -N acumulated over incubation time in soil. Vertical bars represent ± standard error (SE, *n*=3).

3.2 Green house experiment

Results obtained from green house experiment are shown in Fig. 8 and 9. The amounts of NO_2^- + NO_3^--N accumulation were significantly different among the treatments for the soil sampled at 63 and 75 days after sowing. Presence of sorghum showed lower level of NO_2^- + NO_3^--N accumulation over the different incubation time compared with the no plant treatment. At 63 and 75 days after sowing, the inhibition of soil nitrification was varied with incubation period and ranged from 29-69% (Fig. 10). The pH values were significant different (P=0.01) among the treatments (data not shown). Soil without plant showed a lower pH value compared with other treatment.

4 Discussion

In this study, it was shown that nitrification rate in a soil around sorghum root was lower than that the bare soil, although it was not always. This supports the idea that more NH_4^+-N was retained in the soil as a result of nitrification inhibition due to the root exudates of sorghum. Sorghum had been reported to possess inherent ability to release some biochemical compounds from their roots that could inhibit nitrification in soils, a phenomenon known as biological nitrification inhibition (Subbarao et al, 2007a; Hossain et al., 2008). The results of our study showed that the duration of lower nitrification fluctuated from a few days to 3 weeks. Root exudates of sorghum inhibited soil nitrification by 6-85% in fresh soil at 7-14 days after incubation (Figure 7). Using an activity-guided fractionation and purification approach Hossain et al.,2008 isolated one of the active compounds called methyl 3-(4-hydroxyphenyl) propionate, which responsible for inhibitory activity from sorghum root exudates. One of the possible mechanisms through which sorghum may suppress/slowdown nitrifying bacterial populations could be through its root exudates. The suppressive/slowdown effect of soil nitrification would enhance the nitrogen use efficiency, therefore reducing the negative environmental impacts in most agricultural systems due to NO_3^- leaching. Subbarao et al. (2007b) and Subbarao et al. (2007c) reported that NH_4^+-N ions has a stimulatory role in release of biological nitrification inhibition (BNI) from *Brachiaria humidicola* root exudates, however, the exact effects of NH_4^+-N and/or the associated acidic pH that follows after its uptake (i.e. the secondary effect of NH_4^+-N uptake) in release of BNI compounds from plant root have not yet studied

Ammonium application shows promote a significant increase in the levels of accumulated NO_2^-+ NO_3^--N. When nitrification occurs at low pH values, this may be due to the ability of acid-adapted strains of autotrophic nitrifying bacteria to function under this condition.

The brown lowland soil with lower total carbon (6.0 g kg^{-1}) and clay content (20%) has been endowed with less buffer capacity. The buffer capacity play major role in the adsorptive-desorptive soils behavior which might affect the effect of nitrification inhibition by the sorghum. The nitrification inhibitors by the sorghum may be a useful tool for increases N use efficiency, decreasing the N emission and N leaching from soil.

5 Conclusion

Production in situ of nitrification inhibitors, either by plant roots or microorganisms within the rhizosphere of growing crops or pastures, has more appeal as a low-cost alternative to the highly cost synthetic nitrification inhibitors. The results described here showed that nitrification activity in soil around the sorghum root was lower than the bare soil. This suggest that by inhibiting the conversion of ammonium to nitrate, losses associated with leaching and denitrification will be mitigated and economic and environmental benefits will result. This phenomenon and the effectiveness in soils with different chemical/physical properties require more investigation in future.

Acknowledgement

The authors wish to thank Deanship of Scientific Research, King Saud University, College of Food & Agriculture Sciences, and Research Center for supporting this research study.

References

Anonymous. 1974. Technician Autoanalyzer II, Technician Industrial Systems. Tarrytown, NY, USA

Apr DG, Sayavedra-Soto LA, Hommes NG. 2002. Molecular biology and biochemistry of ammonia oxidation by *Nitrosomonas europaea*. Archives of Microbiology, 178: 250-255

Giles J. 2005. Nitrogen study fertilizes fears of pollution. Nature, 433: 791

Glass ADM. 2003. Nitrogen use efficiency of crop plants: physiological constraints upon nitrogen absorption. Critical Review in Plant Science, 22: 453-470

Hossain AK, Subbarao GV, Pearse SJ, et al. 2008. Detection, isolation and characterization of a root-exuded compound, methyl 3-(4-hydroxyphenyl) propionate, sorghum (*Sorghum bicolor*). New Phytologist, 180: 442-451

Litchfield MH. 1967. The automated analysis of nitrite and nitrate in blood. Analyst, 92: 132-136

McCarty GW. 1999. Modes of action of nitrification inhibitors. Biology and Fertility of Soils, 29: 1-9

Prasad R, Power JF. 1995. Nitrification inhibitors for agriculture, health, and the environment. Advances in Agronomy, 54: 233-281

Slangen JHG, Kerkhoff P.1984. Nitrification inhibitors in agriculture and horticulture: a literature review. Fertilizer Research, 5: 1-76

Subbarao GV, Rondon M, Ito O, et al. 2007a. NH_4^+-N triggers the synthesis and release of BNI (Biological Nitrification Inhibition) activity in *B. humidicola* roots. Plant and Soil, 290: 245-257

Subbarao GV, Rondon M, Ito O, et al. 2007b. Biological nitrification inhibition (BNI)-Is it a widespread phenomenon? Plant Soil, 294: 5-18

Subbarao GV, Tomohiro B, Masahiro K, et al. 2007c. Can biological nitrification inhibition (BNI) genes from perennial *Leymus rarcemosus* (Triticeae) combat nitrification in wheat farming? Plant and Soil, 229: 55-64

Subbarao GV, Ito O, Sahrawat KL, et al. 2006a. Scope and strategies for regulation of nitrification in agricultural systems. Critical Review in Plant Science, 25: 303-335

Subbarao GV, Ishikawa T, Ito O, et al. 2006b. A bioluminescence assay to detect nitrification inhibitors released from plant roots: a case study with *Brachiaria humidicola*. Plant and Soil, 288: 101-112

Varley JA. 1966. Automated method for the determination of nitrogen, phosphorus and potassium in plant material. Analyst, 91: 119-126