



## STUDY OF BACTERIAL NITRIFICATION IN SOILS SUBJECTED TO DIFFERENT TILLAGE SYSTEMS

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### SYNOPSIS

**Key words:**  
bacteria,  
nitrification,  
soil,  
tillage systems,  
fertilization,  
unfertilization.

The aim of this study was to determine the nitrifying microbiota involved in the biogeochemical cycle of the nitrogen. The study was designed to investigate the way in which the fertilization and the application of some variants of soil farming influence the nitrifying microbiota. The microbiological determinations, made on 20 samples of fertilized and unfertilized soil, in two ecological seasons (summer and autumn 2009), shows the fact that the nitrifying bacteria are well represented, with variations determined by the type of soil, farming variant, season and climatic conditions. The performed microbiological study demonstrated the efficiency of fertilization and the essential role of the application of efficient farming methods.

### INTRODUCTION

During the natural nitrogen cycle, the mineral nitrogen ( $\text{NH}_4^+$ ,  $\text{NO}_3^{2-}$ ) is used by plants and incorporated in their structure as cellular components. Dead tissues of vegetable and animal origin are mineralized by proteolysis and ammonification and converted by nitrification into forms readily available to plants (Akkermans & Van Elsas, 1995). Nevertheless, nitrogen losses from the soil as a result of the crop plant harvesting, as well as the additional ones determined by denitrification and levigation are so high that, at least in the case of agricultural soils, exceed the amount of existing available nitrogen (Giri et al., 2005). It is estimated that a yield of wheat of 5000 kg/ha is equivalent to a loss of 120 kg. Similarly, a 50000 kg yield of beet requires 200 kg of N, while the production of 5000 kg of dried biomass/ha/year on a meadow in temperate regions implies a loss of 150 kg of N. These are the reasons for which the soil needs to be enriched in combined nitrogen, either naturally, by biological fixation of the atmospheric nitrogen, or artificially, by addition

of nitrogenous fertilizers. Otherwise, the nitrogen deficiency risks of becoming a limiting factor for the primary production in such ecosystems (Koops & Pommerening-Röser, 2001). The global balance of the biogeochemical cycle of nitrogen is a positive one for the biosphere, in spite of the fact there are leakages from this cycle, not only through denitrification, but also through the settling of a part of the organic substances to form sediments. A positive balance, i.e. the amount of nitrogen fixed is higher than the amount of nitrogen lost, is extremely important because it allows the biomass of the entire biosphere to increase (Corbin & D'Antonio, 2004).

Nitrification is a biological process through which  $\text{NH}_3$  or other reduced forms of inorganic nitrogen resulted during the process of ammonification are oxidized to nitrates, which represent the nitrogen compounds most readily digestible by most plants (Buscot & Varma, 2005). Nitrification includes two successive stages tightly linked, and is carried out by specific, different bacteria (Garrity et al., 2005).

Ammonia-oxidizing bacteria – convert ammonia through oxidation into nitrite. They are Gram-negative and heterogeneous in shape (rods, spirals, coccoid etc.). Some have complex intracellular membrane systems visible in ultra-fine sections when examined with an electron microscope. The best known include: *Nitrosomonas europea*, *Nitrosococcus nitrosus*, *N. oceanus*, *N. mobilis*, *Nitrospira briensis*, *Nitrosolobus multiformis*, *Nitrosovibrio tenuis*.

Nitrifying bacteria (nitrite-oxidizing) – convert nitrite through oxidation into nitrate, and are represented by: *Nitrobacter winogradskyi*, *N. hamburgensis*, *Nitrosococcus mobilis*, *Nitrospira gracilis*, *Nitrospira marina*.

Nitrifying bacteria can be found anywhere in the soil, in freshwater and marine environments, in wastewater treatment plants, or in compost depots.

The presence of oxygen is compulsory for all the species of nitrifying bacteria. The reactions cease immediately in its absence or take place at a slower pace as the oxygen tension decreases (Madigan et al., 2000).

Nitrifying bacteria thrive in environments with pH between 7.5 and 8, but in natural environments they also grow in suboptimal conditions (pH = 6). The optimal growth temperature is 25 – 35°C, the lower limit is 5°C, while the upper is 40°C.

In the soil, humidity acts indirectly on the nitrification process, affecting the degree of aeration. Nitrification represents a phase of exceptional importance in the circulation of nitrogen in nature, because it brings nitrate substances from the environment (soil, water etc.) in the form that is most easily assimilable by the plants (Ward & O'Mullan, 2005). It was observed that, in general, the number of nitrifying bacteria is correlated with the degree of soil fertility, reaching in the very fertile soils up to one million of bacteria per gram of soil, also due to the fact that these soils are farmed and aired (Mamiev et al., 2001).

## SUBJECT AND METHOD OF RESEARCH

The aim of this study was to determine the nitrifying microbiota involved in the biogeochemical cycle of the nitrogen.

### DETERMINING THE NITRIFYING MICROBIOTA

Separately, the presence of nitrous and nitric bacteria was researched through the inoculation with suspensions of soil dilutions of two liquid selective culture media, distributed in haemolysis tubes (Atlas, 2004).

To study the nitrous bacteria, the nitrogen is provided as ammonium sulphate and is read with sulphuric diphenylamine. To study the nitric bacteria, the nitrogen is provided as sodium nitrite and is read with the same reactive, after eliminating the nitrites with urea. Each medium was distributed in haemolysis tubes, 1 ml in each tube. After the sterilization, three tubes of dilution were inoculated with 0.5 ml of suspension of soil dilution per each tube, from  $10^{-1}$  –  $10^{-5}$  (the serial dilution technique). The samples were incubated for 20 days at 28<sup>0</sup>C (Angle et al., 1994).

### Reading the results:

**THE NITROUS BACTERIA:** The presence of nitrates or nitrites was determined by emptying the tubes almost completely, to contain only 1 – 2 drops of medium; in each tube there were added 10 drops of H<sub>2</sub>SO<sub>4</sub> and 10 drops of sulphuric diphenylamine. The positive tubes presented a blue hue, more intense at higher concentrations. The reading was also executed against an uninoculated control sample (Dunca et al., 2004).

**THE NITRIC BACTERIA:** The presence of nitrates was emphasized as in the case of nitrous bacteria, with H<sub>2</sub>SO<sub>4</sub> and diphenylamine, 10 drops each, after having added in each tube 10 mg of urea to eliminate the residual nitrites. The presence of nitrates was translated through the same blue coloration (Dunca et al., 2004).

## RESULTS WITH DISCUSSION

The study was designed to investigate the way in which the fertilization and the application of some variants of soil farming influence the nitrifying microbiota. To reach this objective, there were used 10 samples of fertilized soil and 10 samples of unfertilized soil, to which there were applied different variants of farming: disk harrow, Paraplow plough, Chisel plough + rotary harrow, ploughing to 20 cm and ploughing to 30 cm. The samples were taken in the months of June and October 2009 at two different depths (7-10 cm and 15-25 cm) (Fig. 1).

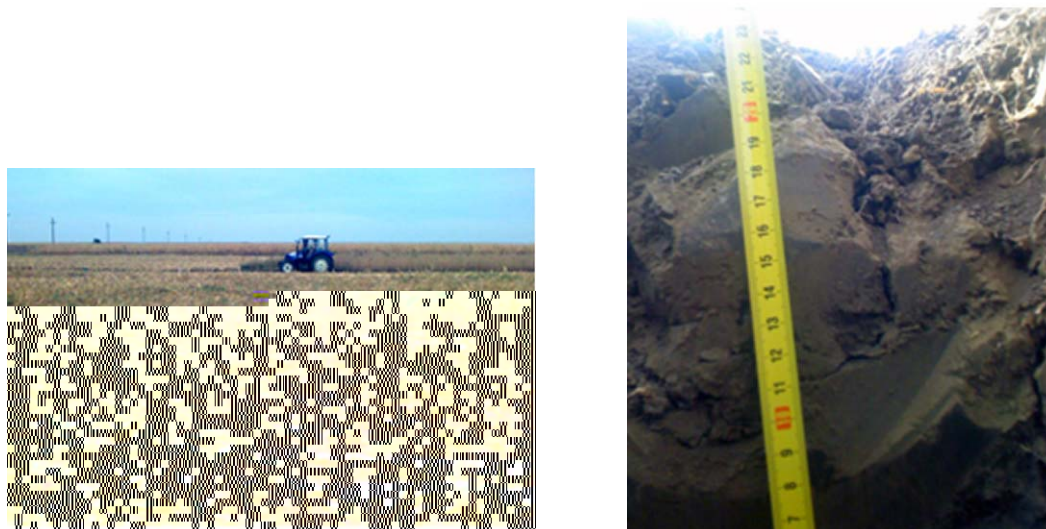


Figure 1: Sampling locations for the soil samples.

The nitrification is a microbiological oxidation process of the ammonium formed after the ammonification, subsequently subjected to the nitrification processes, in which during a first phase, in the presence of nitrite-bacteria, the ammonium is oxidized obtaining nitrite. The nitrification process is directly correlated with the productivity of the soil, it is an important sign of its fertility and is highly influenced by the environmental factors (humidity, temperature, pH, airing etc.).

The nitrifying bacteria (NB) are presented at the level of the superior strata of the soil, where their density is maximum, ensuring the final mineralization process of the organic nitrogen at the level of the substratum. The number of nitrifying bacteria at the level of the analyzed samples is an average of  $10^4$ .

The numerical distribution of the nitrifying bacteria (NB) in the fertilized and unfertilized soil samples is presented in Tables 1 and 2 and Figures 2 - 9.

Table 1: Ecophysiological groups of bacteria involved in the nitrification process in the fertilized and unfertilized soil samples – June, 2009.

Sample no.	Presence/absence of fertilization	Farming variant	Sampling depth (cm)	Conventional notation	No of nitrous bacteria/g soil	No of nitric bacteria/g soil
1.	Fertilized (N80P80)	Disk harrow	7-10	<b>DF1</b>	$43 \times 10^{4+}$	$24 \times 10^4$
2.		Disk harrow	15-25	<b>DF2</b>	$38 \times 10^4$	$22 \times 10^4$
3.		Paraplow plough	7-10	<b>PPF1</b>	$35 \times 10^4$	$24 \times 10^4$
4.		Paraplow plough	15-25	<b>PPF2</b>	$28 \times 10^4$	$25 \times 10^4$
5.		Chisel plough + rotary harrow	7-10	<b>CF1</b>	$36 \times 10^4$	$22 \times 10^4$

6.		Chisel plough + rotary harrow	15-25	<b>CF2</b>	28×10 <sup>4</sup>	18×10 <sup>4</sup>	
7.		Ploughing to 20 cm	7-10	<b>AF1</b>	22×10 <sup>4</sup>	18×10 <sup>4</sup>	
8.		Ploughing to 20 cm	15-25	<b>AF2</b>	43×10 <sup>4</sup>	24×10 <sup>4</sup>	
9.		Ploughing to 30 cm	7-10	<b>AF3</b>	28×10 <sup>4</sup>	22×10 <sup>4</sup>	
10.		Ploughing to 30 cm	15-25	<b>AF4</b>	36×10 <sup>4</sup>	22×10 <sup>4</sup>	
11.		Unfertilized	Disk harrow	7-10	<b>DN1</b>	22×10 <sup>4</sup>	15×10 <sup>4</sup>
12.			Disk harrow	15-25	<b>DN2</b>	18×10 <sup>4</sup>	11×10 <sup>4</sup>
13.			Paraplow plough	7-10	<b>PPN1</b>	28×10 <sup>4</sup>	13×10 <sup>4</sup>
14.			Paraplow plough	15-25	<b>PPN2</b>	15×10 <sup>4</sup>	95×10 <sup>3</sup>
15.			Chisel plough + rotary harrow	7-10	<b>CN1</b>	22×10 <sup>4</sup>	11×10 <sup>4</sup>
16.	Chisel plough + rotary harrow		15-25	<b>CN2</b>	17×10 <sup>4</sup>	76×10 <sup>3</sup>	
17.	Ploughing to 20 cm		7-10	<b>AN1</b>	25×10 <sup>4</sup>	76×10 <sup>3</sup>	
18.	Ploughing to 20 cm		15-25	<b>AN2</b>	28×10 <sup>4</sup>	12×10 <sup>4</sup>	
19.	Ploughing to 30 cm		7-10	<b>AN3</b>	22×10 <sup>4</sup>	84×10 <sup>3</sup>	
20.	Ploughing to 30 cm		15-25	<b>AN4</b>	25×10 <sup>4</sup>	11×10 <sup>4</sup>	

**Table 2: Ecophysiological groups of bacteria involved in the nitrification process in the fertilized and unfertilized soil samples - October, 2009.**

Sample no.	Presence/absence of fertilization	Farming variant	Sampling depth (cm)	Conventional notation	No. of nitrous bacteria / g soil	Nr. of nitric bacteria / g soil
1	Fertilized (N80P80)	Disk harrow	7-10	<b>DF1</b>	54×10 <sup>4</sup>	28×10 <sup>4</sup>
2		Disk harrow	15-25	<b>DF2</b>	43×10 <sup>4</sup>	24×10 <sup>4</sup>
3		Paraplow plough	7-10	<b>PPF1</b>	43×10 <sup>4</sup>	28×10 <sup>4</sup>
4		Paraplow plough	15-25	<b>PPF2</b>	35×10 <sup>4</sup>	28×10 <sup>4</sup>
5		Chisel plough + rotary harrow	7-10	<b>CF1</b>	43×10 <sup>4</sup>	24×10 <sup>4</sup>
6		Chisel plough + rotary harrow	15-25	<b>CF2</b>	36×10 <sup>4</sup>	21×10 <sup>4</sup>
7		Ploughing to 20 cm	7-10	<b>AF1</b>	38×10 <sup>4</sup>	22×10 <sup>4</sup>
8		Ploughing to 20 cm	15-25	<b>AF2</b>	54×10 <sup>4</sup>	28×10 <sup>4</sup>
9		Ploughing to 30 cm	7-10	<b>AF3</b>	35×10 <sup>4</sup>	25×10 <sup>4</sup>
10		Ploughing to 30 cm	15-25	<b>AF4</b>	54×10 <sup>4</sup>	25×10 <sup>4</sup>
11	Unfertilized	Disk harrow	7-10	<b>DN1</b>	25×10 <sup>4</sup>	17×10 <sup>4</sup>
12		Disk harrow	15-25	<b>DN2</b>	21×10 <sup>4</sup>	13×10 <sup>4</sup>
13		Paraplow plough	7-10	<b>PPN1</b>	35×10 <sup>4</sup>	14×10 <sup>4</sup>
14		Paraplow plough	15-25	<b>PPN2</b>	18×10 <sup>4</sup>	11×10 <sup>4</sup>

15	Chisel plough + rotary harrow	7-10	<b>CN1</b>	$28 \times 10^4$	$14 \times 10^4$
16	Chisel plough + rotary harrow	15-25	<b>CN2</b>	$21 \times 10^4$	$95 \times 10^3$
17	Ploughing to 20 cm	7-10	<b>AN1</b>	$28 \times 10^4$	$84 \times 10^3$
18	Ploughing to 20 cm	15-25	<b>AN2</b>	$36 \times 10^4$	$15 \times 10^4$
19	Ploughing to 30 cm	7-10	<b>AN3</b>	$25 \times 10^4$	$84 \times 10^3$
20	Ploughing to 30 cm	15-25	<b>AN4</b>	$28 \times 10^4$	$13 \times 10^4$

Microbiological determinations in samples taken in June, 2009, indicates for the nitrous bacteria, values range between  $15 \times 10^4$  -  $54 \times 10^4$  bacteria/g soil, and for the nitric ones, values between  $76 \times 10^3$  –  $28 \times 10^4$  bacteria/g soil.

In the case of the nitrous bacteria, the numerical increases from one season to the other are not very great, the determined values being close. Thus, it was observed that in June 2009, in the fertilized soils (Fig. 2, Fig.3) the number of nitrous bacteria/g soil oscillates between  $22 \times 10^4$  -  $44 \times 10^4$ . The unfertilized soils (Fig. 4, Fig. 5) presented a lower charge of nitrous bacteria, in some samples, compared to the fertilized ones, with values between  $22 \times 10^4$  bacteria/g soil (the variant: disk harrow, Chisel plough + rotary harrow, ploughing to 30 cm) and  $28 \times 10^4$  bacteria/g soil (the variant: Paraplow plough) – in the case of the samples taken at 7-10 cm; respectively values between  $15 \times 10^4$  (the variant: Paraplow plough) -  $28 \times 10^4$  bacteria/g soil (the variant: ploughing to 20 cm) – in the case of the samples taken at 15-25 cm.

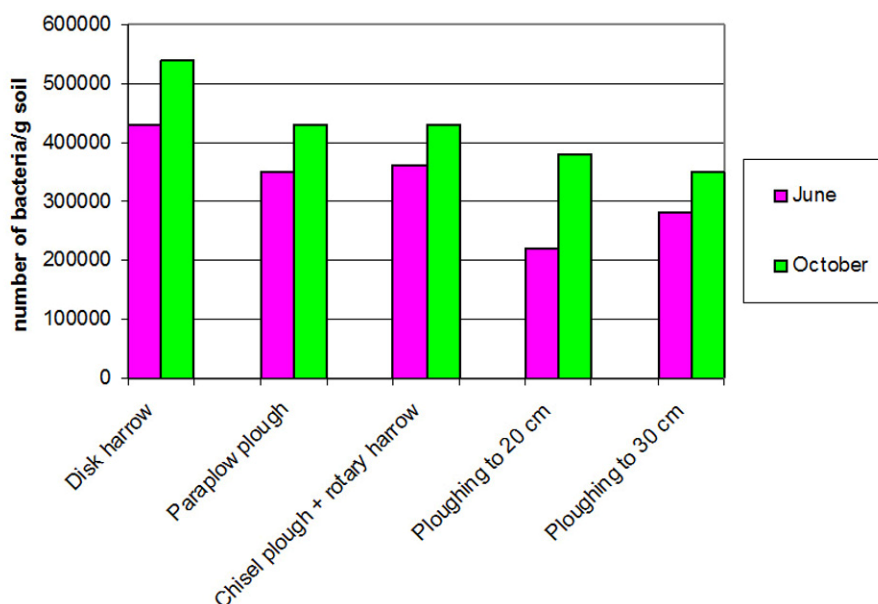


Figure 2: The seasonal distribution of the nitrous bacteria in fertilized soils (7 – 10 cm).

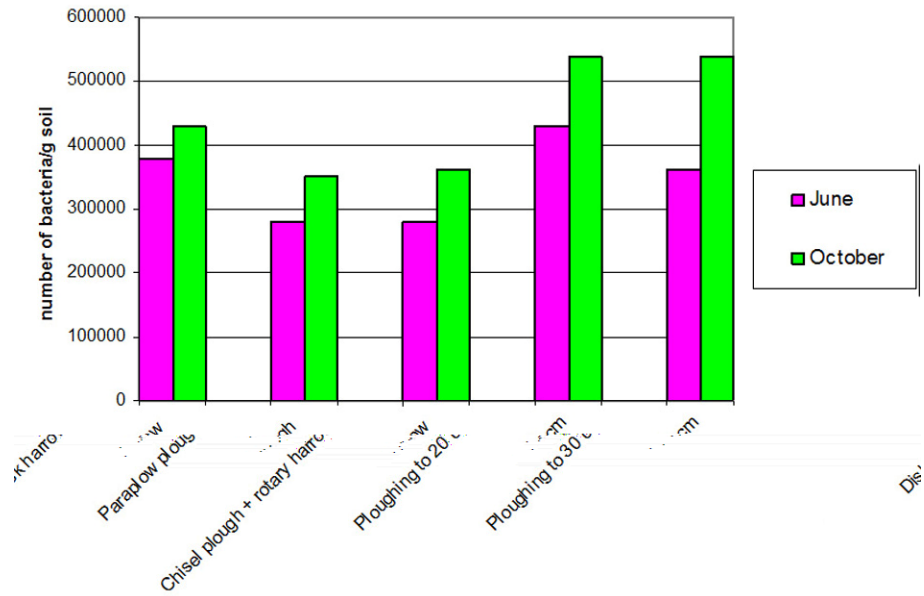


Figure 3: The seasonal distribution of the nitrous bacteria in fertilized soils (15 – 25 cm).

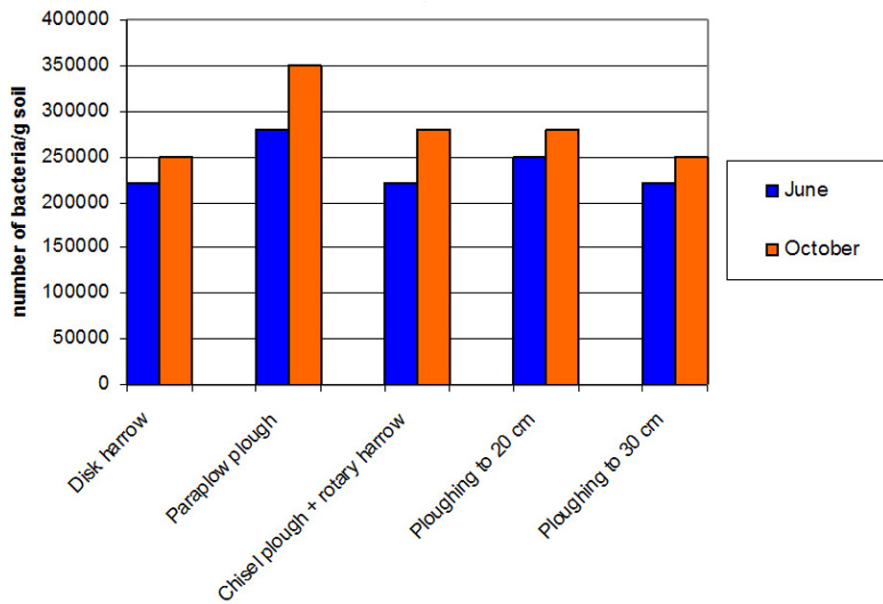
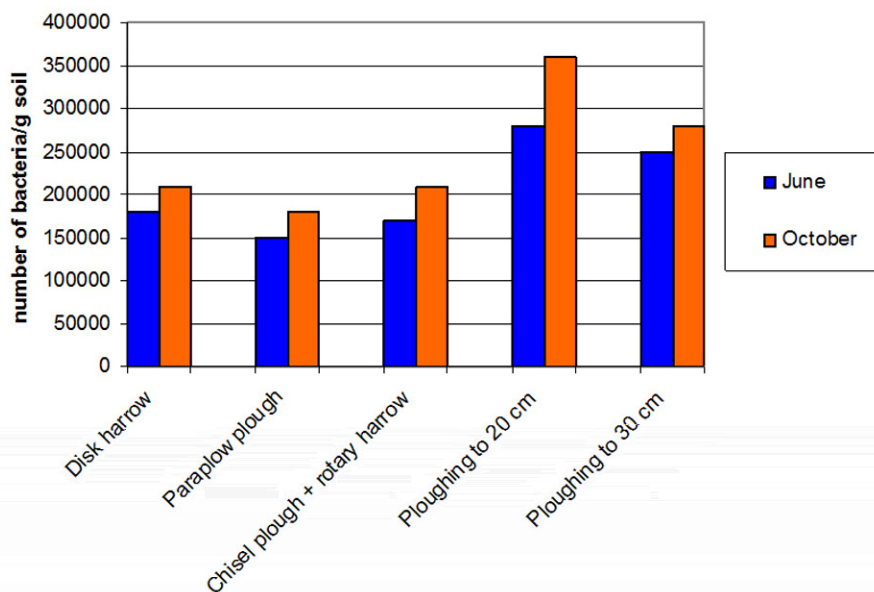


Figure 4: The seasonal distribution of the nitrous bacteria in unfertilized soils (7 – 10 cm).



**Figure 5: The seasonal distribution of the nitrous bacteria in unfertilized soils (15 – 25 cm).**

In October 2009, through the quantitative determinations that were made, it was assessed that the nitrous bacteria presented numerical oscillations between  $35 \times 10^4$  -  $54 \times 10^4$  bacteria/g soil in the case of fertilized soils and  $15 \times 10^4$  –  $36 \times 10^4$  bacteria/g soil in the case of unfertilized soils.

The nitric bacteria were quantitatively less well represented (Figures 6 - 9). For the fertilized soils in June 2009, there were recorded values between  $18 \times 10^4$  bacteria/g soil (the variants: Chisel plough + rotary harrow and ploughing to 20 cm) and  $25 \times 10^4$  bacteria/g soil (the Paraplow plough variant), while in October 2009, the number oscillated between  $21 \times 10^4$  bacteria/g soil (the variant: Chisel plough + rotary harrow) and  $28 \times 10^4$  bacteria/g soil (the variants: Paraplow plough, disk harrow, ploughing to 20 cm).

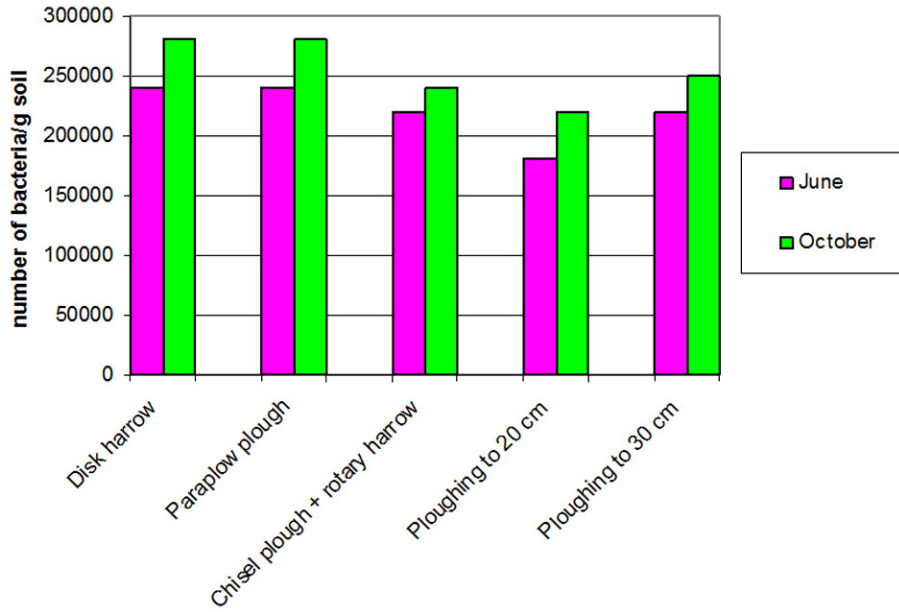


Figure 6: The seasonal distribution of the nitric bacteria in fertilized soils (7-10 cm).

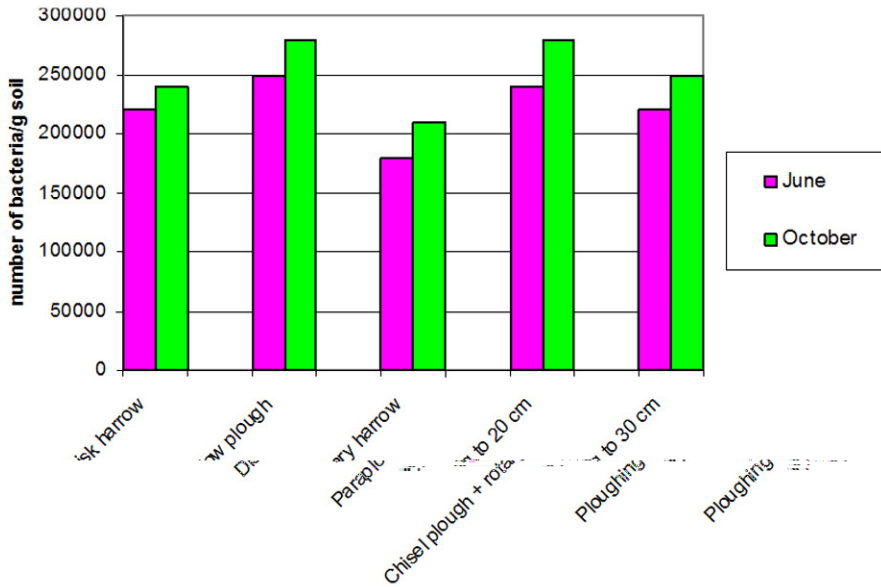


Figure 7: The seasonal distribution of the nitric bacteria in fertilized soils (15-25 cm).

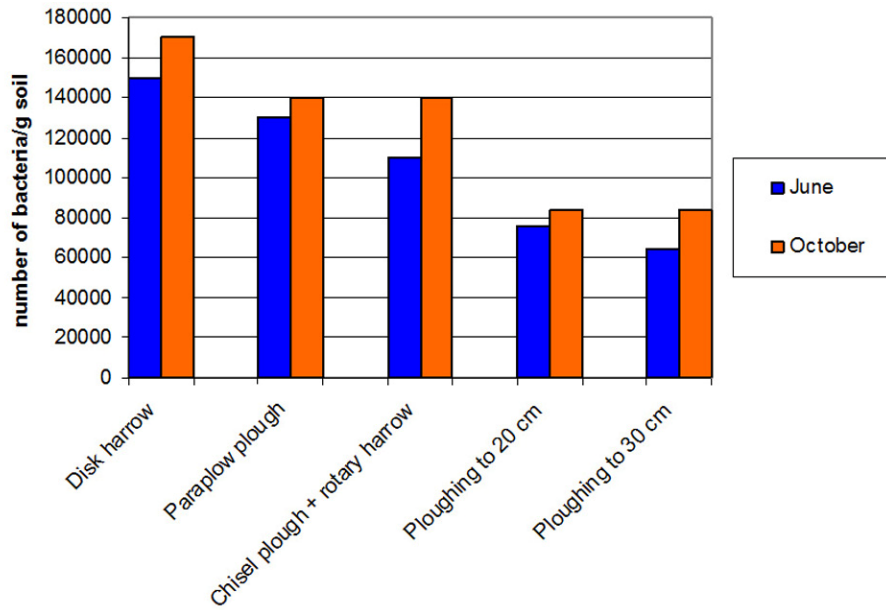


Figure 8: The seasonal distribution of the nitric bacteria in unfertilized soils (7-10 cm).

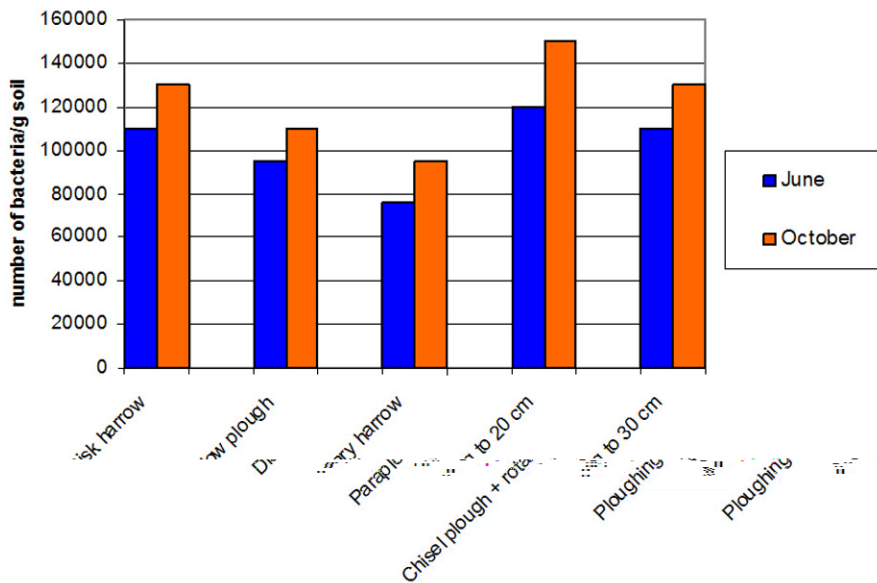


Figure 9: The seasonal distribution of the nitric bacteria in unfertilized soils (15-25 cm).

The lowest values for the nitrifying bacteria (NB) were registered in June 2009 in the samples of unfertilized soil (ploughing to 20 cm – sample taken at 7-10 cm

and Chisel plough + rotary harrow – sample taken at 15-25 cm), where the number of these bacteria/g soil was  $76 \times 10^3$ .

The maximum number of nitrifying bacteria (NB) was recorded in October 2009 in the sample of fertilized soil subjected to the farming variant disk harrow (the sampling depth was 7-10 cm) and ploughing to 20 cm, respectively, ploughing to 30 cm (sampling depth of 15-25 cm), that is  $54 \times 10^4$  bacteria/g soil.

What is worth noticing in these bacteria is their great number in the autumn season, with results comparable to the data in the specialized literature (Cébron et al., 2003), according to which when the quantity of biomass is large the number and the activity of the nitrifying bacteria is also high. Other factors that influence the number of these microorganisms are high temperatures, reduced quantities of  $\text{NH}_3$  and high quantities of nitrates.

In the case of unfertilized soils, the values were lower, the minimum being  $76 \times 10^3$  bacteria/g soil (the variants: Chisel plough + rotary harrow and ploughing to 20 cm) for June 2009, and the maximum of  $15 \times 10^4$  bacteria/g soil – in case of the farming variant– disk harrow (October, 2009).

There can be observed a considerable increase in the number of nitrifying bacteria (NB), an increase caused, probably, by the local ecological conditions (pH, humidity, airing).

Comparing the evolution of the number of nitrifying bacteria (NB) in the fertilized and unfertilized soil samples, from one season to the other, there can be observed that in most samples the number of bacteria increased significantly, being higher in the autumn season. These high values indicate an extremely intense microbiological activity of the nitrifying bacteria in this period of the year.

Studying the seasonal variation, there can be observed the stimulating effect of high temperature in the autumn period on the bacterial populations, which translates in a higher number of bacteria in most soil samples that were studied.

The numerical differences between the two seasons vary between rather narrow limits, as the high values recorded in October 2009 are probably also caused by the accumulations of vegetal and animal organic matter at the end of the vegetation period, demonstrating the existence of relatively high quantities of particulate nitrate substratum, which stimulates the development of microorganisms.

The numerical differences of the nitrifying bacteria (NB) recorded in the soil samples analyzed are caused not only by the farming variants, but also by the impact of the fertilization phenomenon.

The favourable effect of autumn works impacts on the cultivated plants, as well as on the activity of microorganisms in the soil. Following the analysis of samples taken from plot ploughed in the autumn, comparatively to those ploughed in the spring (Koops & Pommerening-Röser, 2001) it was observed that in the autumn variant, the activity of the nitrifying bacteria is better. The increase of the intensity of

the nitrification is correlated with the best degree of soil loosening and soil humidity, which in its turn favours this process.

## CONCLUSIONS

1. The microbiological determinations made on 20 samples of fertilized and unfertilized soil, in two ecological seasons (summer and autumn 2009) shows the fact that the nitrifying bacteria are well represented, with variations determined by the type of soil, farming variant, season and climatic conditions.

2. The nitrification, the microbiological process directly correlated with soil fertility, is different in the analyzed soil samples. The most intense nitrifying activity was emphasized in the fertilized soils (the variants: disk harrow, ploughing to 20 cm and, respectively, to 30 cm) in October 2009, and the lowest one, where the microbiological processes developed slower, in the unfertilized soils, in June 2009.

3. Temperature, organic substances, fertilization and the farming variants have an important role in the development of the microbial populations, influencing the numerical values recorded during the two seasons of sampling.

4. The high values characterize the autumn season, the causes being the high temperatures during that period of the year and the increased input of vegetal and animal organic matter at the end of the vegetation period.

5. The performed microbiological study demonstrated the efficiency of fertilization and the essential role of the application of efficient farming methods (for example: the disk harrow or the ploughing to 20 cm).

## ACKNOWLEDGEMENTS

The financial contribution to this work from the Romanian Ministry of Education, Research, Youth and Sport, National Center for Program Management, program Partnerships in priority areas „Soil and water resources management in agroecosystems affected by drought excessive in order to maintain biodiversity (MOLDOTECH)” no. 51-017/ 14.09.2007, is gratefully acknowledged.

## REFERENCES:

- AKKERMANS, A.D.L. & VAN ELSAS, J.D. 1995: Molecular microbial ecology manual. - *Kluwer Academic Publishers, Dordrecht The Netherlands*, 488 pp.
- ANGLE, S., WEAVER, R.W., BOTZTOMLEY, P., BEZDICEK, D., SMITH, S., TABATABAI, A. & WOLLUM, A. 1994: Methods of soil analysis, part 2 – Microbiological and biochemical properties. – *Soil Science Society of America, Inc.*, 1692 pp.

- ATLAS, R.M. 2004: Handbook of Microbiological Media. 3<sup>rd</sup> edition. - *CRC Press, New-York*, 464 pp.
- BUSCOT, F. & VARMA, A. 2005: Microorganisms in soils: roles in genesis and functions. - *Soil Biology, Springer*, 3: 165-168.
- CÉBRON, A., BERTHE, T. & GARNIER, J. 2003: Nitrification and nitrifying bacteria in the lower Seine River and estuary (France). - *Applied Environmental Microbiology*, 69 (12): 7091-7100.
- CORBIN, J.D. & D'ANTONIO, C.M. 2004: Effects of exotic species on soil nitrogen cycling. Implication for restoration. - *Weed Technology*, 18(1): 1464-1467.
- DUNCA, S., AILIESEI, O., NIMIȚAN, E. & ȘTEFAN, M. 2004: Microbiologie aplicată. - *Tehnopress Publishing, Iași*, 293 pp.
- GARRITY, G.M., BRENNER, D.J., KRIEG, N.R. & STALEY, J.T. 2005: Bergey's Manual of Systematic Bacteriology, 2<sup>nd</sup> edition, vol.2 – The Proteobacteria. - *Springer Verlag, New York*, 1388 pp.
- GIRI, B., GIANG, P.H., KUMARI, R., PRASAD, R. & VARMA, A. 2005: Microbial diversity in soils. In: Buscot, F. & Varma, S. (Eds), Microorganisms in soils: roles in genesis and functions. - *Springer Verlag, Heidelberg, Germany*, pp: 195-212.
- KOOPS, H.P. & POMMERENING-RÖSER, A. 2001: Distribution and ecophysiology of the nitrifying bacteria emphasizing cultured species. - *FEMS Microbiology Ecology*, 37: 1-19.
- MADIGAN, M.T., MARTINKO, J.M. & PARKER, J. 2000: Brock Biology of Microorganisms. - *Pretince Hall, Inc. Upper Saddle River, New Jersey*, 300 pp.
- MAMIEV, M., EGAMBERDIYEVA, D. & POBEREJSKAYA, S.K. 2001: The influence of mineral fertilizer combined with a nitrification inhibitor on microbial population and activities in calcareous Uzbekistanian soil under cotton cultivation. - *Scientific World Journal*, 1 (2): 108-113.
- WARD, B.B. & O'MULLAN, G.D. 2005: Relationship of temporal and spatial variabilities of ammonia-oxidizing bacteria to nitrification rates in Monterey Bay, California. - *Applied Environmental Microbiology*, 71 (2): 697-705.

Original research article

Received: 20 July 2010

