THE BEHAVIOR OF NITRIFYING MICROORGANISMS FROM A SOIL CULTIVATED WITH *LOTUS CORNICULATUS* L.

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Abstract
The knowledge of the behavior of the nitrifying microorganisms is of real use, because the microbial activities related to the nitrogen circuit and especially the nitrification determine the results of the agronomic practices. In this paper, the influence of the *Lotus corniculatus* L. (I-IV culture years) on the nitrifying bacteria and the correlation between the growth of these bacteria and soil moisture was studied.The soil samples were taken during the flowering period (summer season), from the rhizosphere of the pottery, but also from the uncultivated plots (control), placed in the western part of the country. Studies have shown that plants and soil moisture have influenced the evolution of nitrifying bacteria.

Key words: nitrifying bacteria, *Lotus corniculatus* L., rhizosphere, *Nitrobacter, Nitrosospira*

Introduction
Nitrogen is one of the most important nutrients for plant growth, limiting the primary productivity in many terrestrial ecosystems. For this reason it is applied in large quantities in agricultural systems, in the form of chemical fertilizers. The dynamics of this chemical element depends on some key microbial activities, such as: molecular nitrogen fixation, ammonification, nitrification, denitrification. Through nitrification, the conversion of ammonium to nitrites, is essential for soil microbiota and plant nutrition. Oxidation of ammonia to nitrites is accomplished by bacteria and arhebacteria, and oxidation of nitrites to nitrates is the result of nitrate-bacterial activity [1]. There are two groups of bacteria involved in the nitrification process (*Nitrobacter* and *Nitrospira*).The increase of agricultural production by applying chemical fertilizers results in the loss of significant amounts of nitrogen through leaching [6], a process followed by eutrophication of water with an important effect on biodiversity. It also produces emission of nitrogen oxide, greenhouse gas, which contributes to global warming of the Earth [2]. Therefore the knowing of this process provides important information on nitrogen availability, an essential element for agricultural crops and soil livestock. As a result, researchers use alternatives that could reduce these unwanted effects, including growing leguminous plants [15].The positive effects of legume cultivation lie in the fact that, if nitrogen loss is reduced, the nitrifying microorganisms would not be blocked by the nitrogen introduced through the anthropic pathway and would not affect the availability of nitric nitrogen for large nitrogen-consuming crops. The same researchers point out that the process of nitrification depends on the composition of the nitrifying community. It is also appreciated that the structure of this bacterial segment is influenced by soil moisture, water being an essential factor for the development of enzymatic activity [7,9] appreciate that the thermal and hydrological regime are the main factors responsible for the evolution of the microbiological and biochemical parameters of the soil. The rhizosphere is the soil that surrounds the roots system, through which plants release a large amount of metabolites that act on the one hand as chemoreceptors for microorganisms, and on the other hand they are essential nutrients for plant growth [5,12]. Based on all of this information in an

initial step we evaluated the abundance of nitrifiers from the soil cultivated with pods in relation to the uncultivated soil and followed the effect of soil moisture on the nitrifying bacteria. We consider that these results will provide a viable ecological alternative that will in turn provide the necessary nitrogen for plants, without loss and without affecting the nitrifying microbial flora. It should be mentioned that nitrification is particularly important for soil fertility.

Materials and methods

Our studies were performed on a soil from the perimeter of Arad County, which is located between 20045'- 22030 'east longitude and 45 0 58'- 460 38' north longitude, with a moderate continental temperate climate and soils consisting mostly of sedimentation parental materials [20]. Soil samples were collected from the root growth depth of *Lotus corniculatus* L., from the rhizosphere area (Rh1-Rh4), but also from the uncultivated plots, used as a control (Mt1-Mt4), during the flowering period (the growing season - summer). The soil from 4 years of culture was microbiologically analyzed in the same calendar year.

The soil samples processing and the setting up of experiments was carried out in the Microbiology laboratory, University of Agricultural Sciences and Veterinary Medicine of the Banat "King Michael I of Romania" in Timisoara and was accompanied by the determination of humidity by using the thermo-gravimetric method, with Sartorius scale MA-50 at 105°C, as described by Bordean et al. 2011 [3]. Isolation of nitrifying bacteria from soil samples was performed on liquid mineral nutrient medium, supplemented with color indicator. The principle of the method was according with Saratchandra, taken over by Stefanic (2006) . The growth temperature of the bacteria was 28°C with an incubation time of 8 weeks.

Results and discussions

The availability of nitrogen from the soil influences the abundance and composition of nitrifiers. Nitrifying bacteria occupy different ecological niches and their abundance varies with the amount of ammonium in the soil [14].

Nitrifying bacteria were found in large numbers in the control plots during the first 2 years. The number of nitrifiers decreased with the passage of years; the number of nitrifiers was lower in the years III- IV and the smallest number was highlighted in the control version from year IV.The highest number of nitrifying bacteria was isolated from the rhizosphere of the culture from the year II, compared to the other experimental variants. Depending on the abundance of the nitrifiers the experimental variants follow one another in descending order: Rh2>Rh1>Rh3>Rh4.

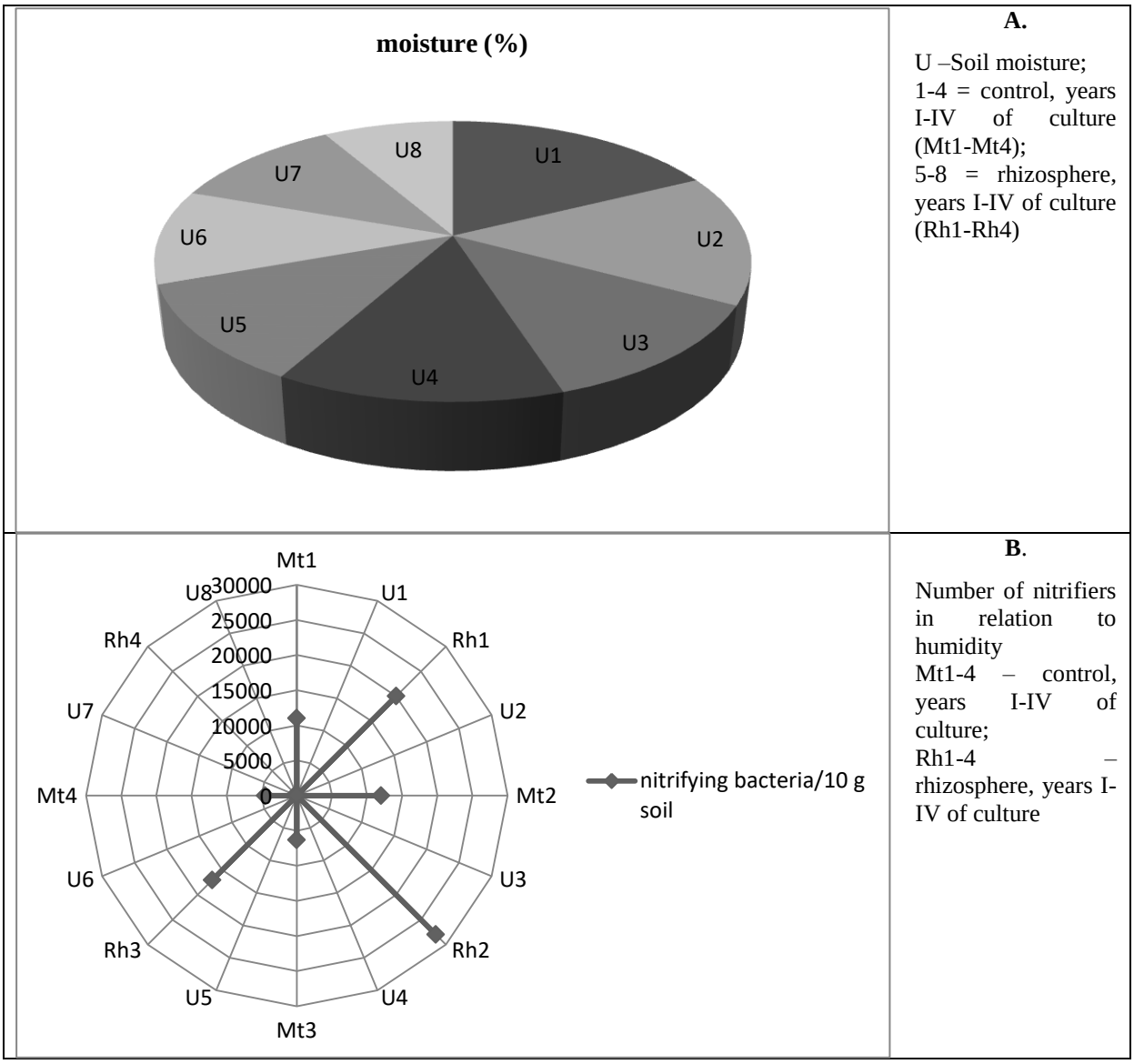

Figure 1 The abundance of nitrifying bacteria from experimental variants depending on soil moisture (%)

Compared to the control variants, it was observed that an increase of the nitrifiers in the rhizosphere of plants, in the I-III years of culture, except for year IV when the number of bacteria in the rhizosphere (Rh4) was lower compared to the control (Mt4). Plants have influenced nitrifying bacteria, as demonstrated by many studies [13, 18]. Another important factor that can determine the variation of the bacterial community is the vegetation type. According to Attard et al., 2010, nitrification depends on the methods of processing and treating the soil [1], but also on the changes in the genera *Nitrosospira* and *Nitrobacter*.

The soil moisture from the experimental variants is high in the Mt1 variant, followed by Rh1, Rh2 and the other experimental variants (Figure 1A). The samples with the lowest water content come from Rh4, where the lowest number of nitrifying bacteria was recorded (Figure 1B).

Humidity influences the evolution of nitrifiers in the soil, but the plant also makes a significant contribution, as the evidence that the largest number of nitrifying bacteria is found in soil samples from the rhizosphere of plants (fig. 3,4B). The results are in concordance with some authors who report that excessive soil moisture causes low microbial biomass [17], and

low water content disrupts soil homeostasis. In contrast, [4] observed that soil moisture influenced soil microbial and biochemical activity to a lesser extent than soil type. Other studies have shown that the microbial and biochemical activity of the soil is closely related to the physical and chemical properties of the soil [10].

Conclusions

Soil microbial activities are determined by a variety of factors, including moisture and the plant. This influence is seen in the evolution of nitrifying bacteria from the rhizosphere of Lotus*corniculatus* L. plants in the first years of culture. The highest number was recorded in the rhizosphere of the plants of the second year followed by the rhizosphere of the first year of the culture. The smallest number of nitrifiers was highlighted in the rhizosphere of plants from the fourth year of cultivation.The number of nitrifiers in the 8 experimental plots decreased simultaneously with the year of culture and the water content.

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