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# THE STUDY OF THE BACTERIAL COMMUNITY IN THE ROOT AND NEAR-ROOT ZONE OF SPECIES VICIA SATIVA

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***Abstract:*** *By using crop rotation and introducing legume crops, farmers can improve the structure of the system and change soil characteristics for the better. At the same time, the bacterial community is stimulated and enzyme activity is intensified, while an optimal nutritional regimen is created. Another improvement that comes from growing legumes is that it creates conditions for the accumulation and storage of soil humidity (INSAM AND DOMSCH 1988; WARDLE 1992, KOWALCHUK ET AL, 2002, SHARMA1, ET AL, 2005). The aim of the paper is to study the way in which species Vicia sativa, organic matter and soil humidity index can influence the structure of the bacterial community in moderately-gleyed eutric cambisol. Ten samples of soil were collected as biologic material from two experimental fields sown with Vicia sativa, which is going to be incorporated into the soil as "green fertilizer". The biologic material was processed and conditioned at the Microbiology Laboratory at Banat University of Agricultural Sciences and Veterinary Medicine, Timisoara. The microorganisms in the root area and near-root area (experiment I and II) were isolated on YMA growth medium, using the plate culture method (BOROZAN, 2006). In parallel we looked for the presence of bacteria specific for legumes and root node bacteria belonging to genus Rhizobium (experiment I). We noticed changes in the microbial balance in the rhizosphere and rhizoplane. These changes were characterized by increases of UFC/g soil, the largest number being recorded on the rhizoplane (in experience II in particular). In this last variant, the microbial segment is the most reduced, which proves the fact that quantitative changes in the microbial balance in the root and near-root zone of the soil is determined by the substances eliminated by vetch into the soil. Of the two experiments, the second experience gives the highest number of UFC/g soil. The statistical data revealed a certain contribution of the humidity index and humus content in the evolution of UFC/g soil. In what the formation of root nodules on the root of Vicia sativa is concerned, when the plant grows on slightly gleyed eutric cambisol (1st experiment), we noticed numeric variation of these, for similar values of the humidity index and humus content.*

**Key words:** *bacterial community, rhizosphere, rhizoplan, Genus Rhizobium, Vicia sativa*

## INTRODUCTION

Although the degree of microbial biodiversity is not known, the soil is the biggest reservoir of biological diversity (DYKHUIZEN et al, 1998). Once vegetation appears and the root develops in the soil, another type of microbial balance settles in, around the roots, where a great number of microflora can be noticed (STEFANIC AND SANDOIU, 2011).

Researchers have rightly been focussing on the study of the correlation between plant diversity and composition and the microbial biodiversity in the soil. The implications of this relation on the stability, resistance and functioning of ecosystems are equally important (KOWALCHUK et al, 2002). The results obtained by the same authors show that microbial colonization of the rhizosphere is maintained throughout vegetation. The plants are considered to be one of the factors with profound effects on microbial communities and processes, especially in the rhizosphere (INSAM AND DOMSCH 1988; WARDLE 1992). It is well-known that there are mutual interactions between legumes and bacterial species of genus *Rhizobium*; these interactions are materialized in the appearance of root nodosities and the realization of a link in the bio-geo-chemical cycle of nitrogen (HIRSCH,et al 1992). The "virulence" is determined by different sets of genes found both in the host plant and in bacterial symbionts (VERMA, et al,. 1992; WIEHE, et al, 1994.)

Making use of molecular techniques, researchers have shown the microorganism-plant dependence, as well as significant differences in the structure of the bacterial communities of three legumes: *Vicia faba L., Pisum sativum L., and Lupinus albus L* (SHARMa, et al, 2005 ).

*Rhizobium* bacteria are widely spread in arable soil, in the rhizosphere of legumes and non-legumes (wheat, oats and maize) (CHELIUS AND TRIPLETT, 2001; CLARK et al., 2002; HILALI et al., 2001; SCHMALENBERGER et al., 2001; SCHMALENBERGER et al., 2003). Even though the diversity and composition of the natural populations of *Rhizobium* in symbiotic interactions have been studied extensively in our country and abroad (AMARGER, 2001), little information is available on the factors which influence them in the soil.

This paper studies in detail the way in which species *Vicia sativa*, organic matter and soil humidity degree (in the case of moderately-gleyed eutric cambisol) can influence the structure of bacterial communities.

## MATERIAL AND METHODS

We took the ten soil samples (experiment I and II) and the respective common vetch plants from Banat area in autumn 2010. Besides these samples we also took one control sample. The biologic material was processed and conditioned at the Microbiology Laboratory from Banat University of Agricultural Sciences and Veterinary Medicine, Timisoara. The microorganisms in the root area and near-root area (experiment I and II) were isolated on YMA growth medium, using the plate culture method (BOROZAN, 2006). In parallel we looked for the presence of bacteria specific for legumes and root node bacteria belonging to genus *Rhizobium* (experiment I). The growth conditions were the ones in use for mesophilic cultures. The incubation lasted for 48 hours.

### *Statistical analysis*

The statistical evaluation of the experimental data was made using PAST 2.14., as well as MVSP 3.1 and PAST 2.14.

Principal Components Analysis (PCA) is a mathematical model that enables researchers to identify patterns in data by “expressing the data to highlight their similarities and differences” (SMITH, 2002, HAMMER et all, 2001).

Cluster Analysis is a statistical method that groups data objects based on information found in the data that describes the objects and their relationship (http://www-users.cs.umn.edu/~kumar/dmbook/ch8.pdf).

The cluster analysis was performed using Two-way clustering using Ward’s method based on the Euclidian distances between all members in the two groups. In Ward's method, clusters are joined in such a way that increase in within-group variance is minimized.

## RESULTS AND DISCUSSION

The data we obtained were processed statistically and their graphic representation is presented below (Figures 1 to 4).

In the presence of the same humidity coefficient and of similar humus content, we noticed variation in the number of root nodosities (Figure 1). The formation, number and size of nodosities is not determined only by the virulence of bacteria, but also by plant reaction and the conditions provided by the environment at the time (STEFANIC, 2011).

Vicinity was observed among plant groups $A_{3-6,\ 8-9}$, with small differences between them. Plant $A_7$ ranked close to them, and plants $A_{1-2}$ and $_{10}$ formed a distinct group, although differences can be noticed among the individuals in this group also. (Figure 2).

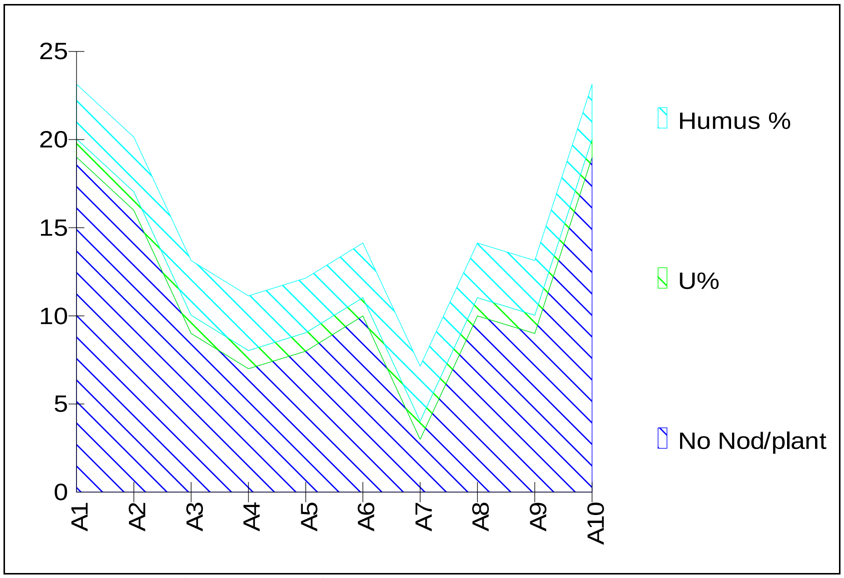


Figure 1. Variation of the Number of Root nodosities/ Plant with Humus % and Humidity % (I)

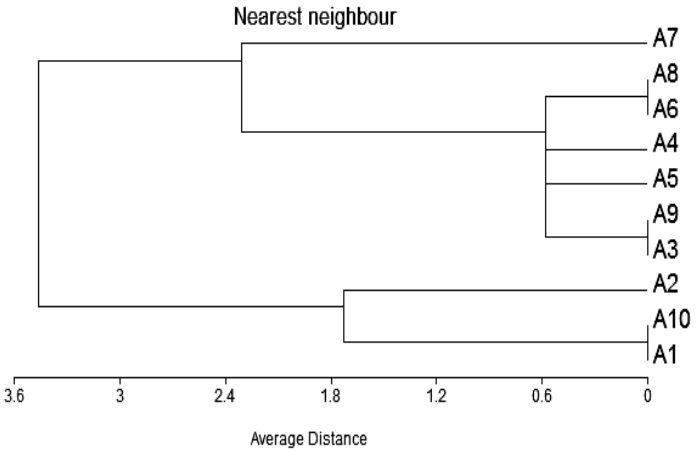


Figure 2. Cluster Analysis Representation of the average distances between the studied plants using Nearest Neighbour equation (I)

Under the conditions of different contents of humus and humidity, cluster analysis highlights the microbial zones in three neighbour groups: control variants I, II; rhizosphere areas I, II and root areas I, II (Figure 3b).

UFC-$D_1$/g soil is influenced by the humus content. UFC-$D_2$/g soil is influenced by the humidity coefficient (Figure 3 a,b).

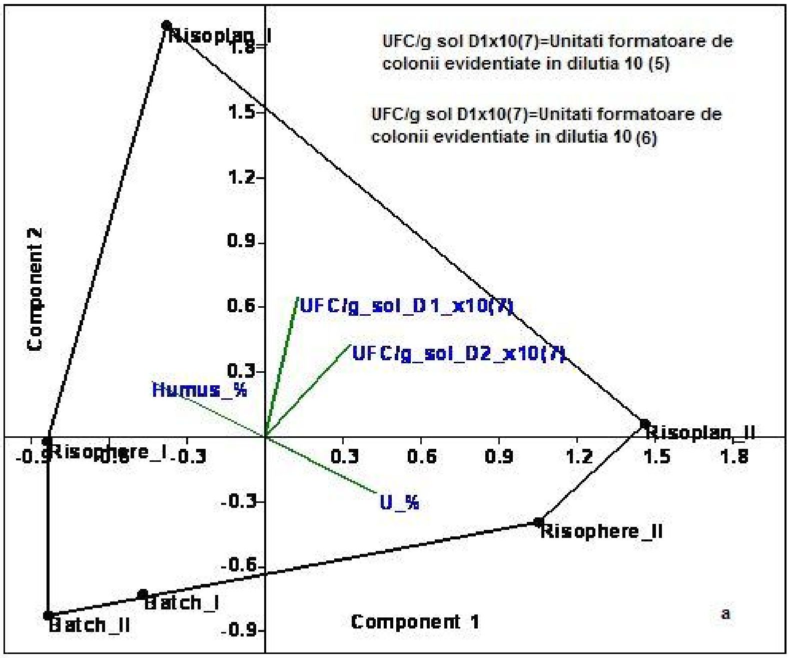
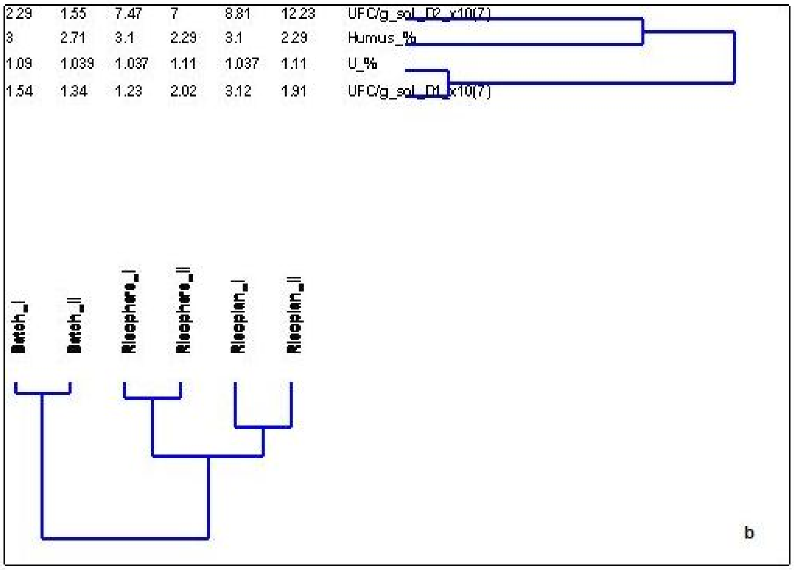


Figure 3. a. Scatter representation of PCA using PC1 axis



| | | | | | | |
|---|---|---|---|---|---|---|
| 2.29 | 1.55 | 7.47 | 7 | 8.81 | 12.23 | UFC/g_sol_D2_x10(7) |
| 3 | 2.71 | 3.1 | 2.29 | 3.1 | 2.29 | Humus_% |
| 1.09 | 1.039 | 1.037 | 1.11 | 1.037 | 1.11 | U_% |
| 1.54 | 1.34 | 1.23 | 2.02 | 3.12 | 1.91 | UFC/g_sol_D1_x10(7) |

Figure 3. b. Two way Cluster Analysis Representation using Ward’s method, Euclidian distances (I,II)

Specialty literature provides data that show a change in the structure of bacterial communities found in non-agricultural plants; these changes are brought about by soil-plant interaction and soil-microorganism interaction. In their turn, changes in the structure of microbial community can have important implications in plant growth, vegetation succession and other vital processes that take place in the soil (KOWALCHUK et al, 2002; BEVER 1994). It has been theorized that bacterial diversity is great in isolated places with low carbon content, found at soil surface (ZHOU et al. 2002).

The % variance on PC1 is 49.721 and on PC2 it is 33.523, so we choose to represent the eigenvalues only on the most significant axis PC1 (Figure 3a).

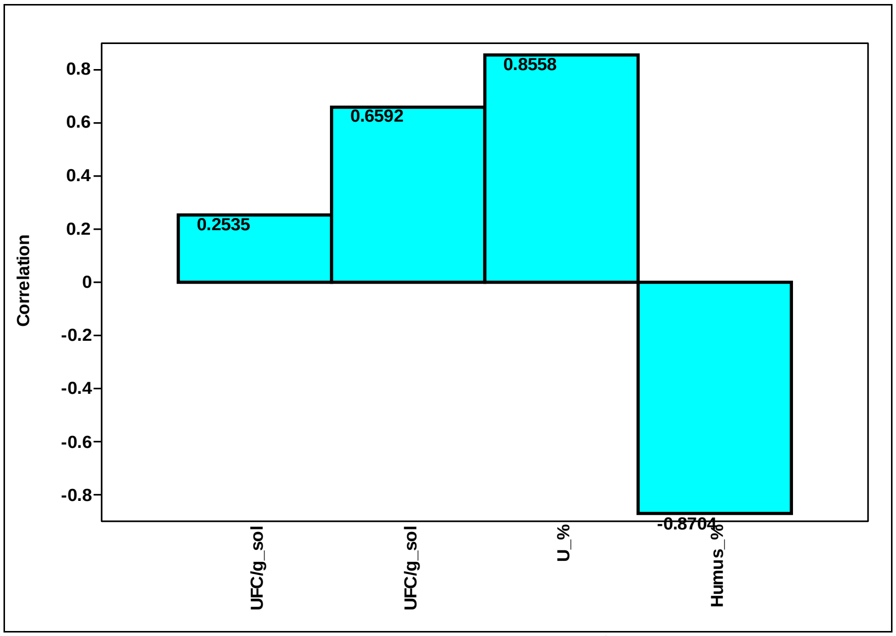


Figure 4a. Variables correlation loadings for PC1

When compared to axis PC1, where we find positive correlations between UFC/g soil and humidity coefficient, but negative in the case of humus, on axis PC2 these relations are inverse (Figure 4.a,b).

## CONCLUSIONS

The microfloras in the biotopes found around rootlets are more numerous than the ones in root area and in the control variant. In the latter, the microbial segment is the leat numerous, which proves the fact that quantitative modifications of the microbial balance in the rhizospheric or respectively in the root area of the soil is determined by the substances that the common vetch releases in the soil.

Of the two experiments, we found the greatest number of UFC/g soil in experiment II, root area, followed by the rhizospheric area. Based on the statistical analyses, we found a certain contribution of the humidity coefficient and of the humus content in the evolution of UFC/g soil.

Bacteria of genus *Rhizobium* are gram-negative soil bacteria which have profound significance for science and agriculture, because of their ability to establish symbiosis with legumes for nitrogen fixation, which plays a major role in maintaining soil fertility. In what virulence is concerned, although we only studied it in experiment I, we can state that it is variable under similar humidity conditions and for the same content of organic matter.

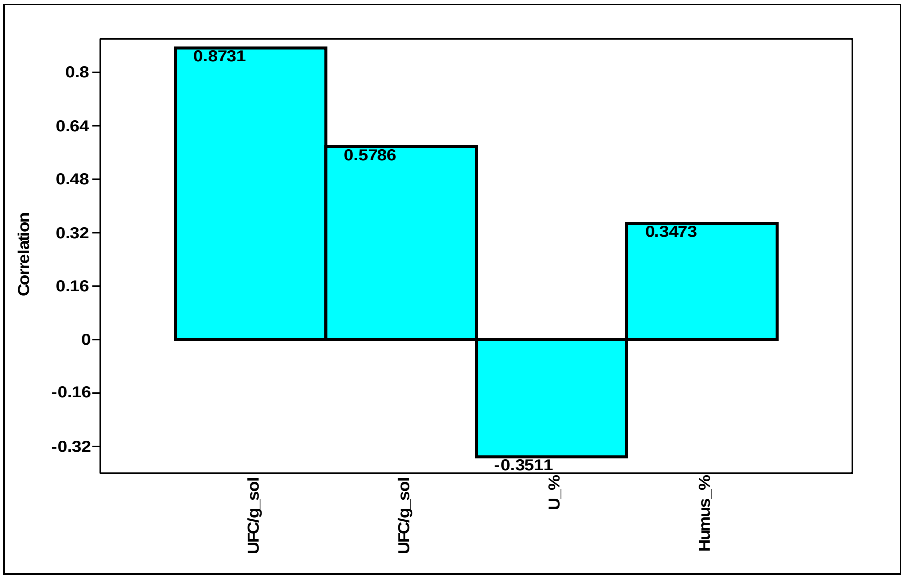


Figure 4b. Variables correlation loadings for PC2

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