OSCILLATION INDUCED BY AGRICULTURAL INPUTS IN MICROBIAL COMMUNITIES FORMED IN SOYBEAN RHIZOSPHERE

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Abstract

Rhizosphere is the area with the most intense microbial activity, under the direct influence of plant species and soil type. Changes in activity and functional diversity of microorganisms can represent powerful parameters in the analysis of soil quality. Usage levels of carbon sources by functional groups of microorganisms is found in the changes of microbial community structure, crop plants stimulating the activity of specific groups. This paper aims to assess the oscillations of activation / inactivation of microbial functional groups specific in the rhizosphere of soybean, correlating the activity of whole microbial community with the amount of applied agronomic inputs. Soybean plants were grown on a phaeosiom argic in the hilly area of Transylvania, experimental field being located in Turda, Cluj county. Detection plates of microbial activity were inoculated with soil according to the method Microresp and incubated for a period of 6 hours. Typical soil microbial community present in soybean plants is dominated by α-Ketoglutaric acid. Codominant of this group in the community are decomposers of D-fructose, L-malic acid and citric acid. Microbial functional dynamics in unfertilized soil is dominated by microorganisms involved in the nitrogen cycle, enhancing the accumulation of soil organic nitrogen. Microorganisms from the rhizosphere of soybean crop have a strong reaction to associated fertilization, zeolite acting as a buffer for disruption caused by the mineral component of fertilizer recipes.

Key words: soybean, rhizosphere, microbial communities, substrate decomposition

Sustainable agriculture is connected with the quality of soil and plant potential to develop a complex rhizosphere (Das B., Chakrabarti K., 2013). Rhizosphere is the interface between plant and soil with the highest intensity of microbial activity and nutrient exchanges (Mendes L.W. *et al*, 2014). Plant rhizosphere is responsible for regulating agro-ecosystem stability (Ehrmann J., Ritz K., 2014). Microflora in this area is extremely sensitive to changes in the level of nutrients and their flow (Griffiths B.S. *et al*, 2007). Microbial diversity is responsible for the speed and dynamics of processes in the rhizosphere, stimulating plant growth (Yang Q. *et al*, 2013). Available carbon sources are a good indicator of functional specialization of microorganisms located in rhizosphere (Garland J.L., Mills A.L., 1991), ensuring the fertility of the soil.

Current agriculture needs to increase soil fertility in order to achieve a high level of sustainability and consistency of production. Due to the importance of microbial communities in processes of growth and development of plants, microbial functional dynamics is analyzed and correlated with soil fertility (Sugiyama A. *et al*, 2014, Trabelsi D., Mhamdi R., 2013). Potential productions are easier to be predicted based on the presence of microbial communities and their efficiency in the conversion of soil nutrient reserves (van Ittersum M.K. *et al*, 2013).

MATERIAL AND METHOD

The experiments were located at ARDS Turda on a phaeoziom argic soil (46°35'31.4"N 23°48'19.8"E). On each variant a treatment with zeolite, urea, NP 20-20 or various combinations of these have been applied. The biological material is soybean (Glycine max L.). Experimental variants were: V1 – control (unfertilized), V2 – 100 kg ha^{-1} urea, V3 – 100 kg ha^{-1} zeolite, V4 – 50 kg ha^{-1} urea + 50 kg ha^{-1} zeolite, V5 – 70 kg ha^{-1} urea + 30 kg ha^{-1} zeolite, V6 – 50 kg ha^{-1} NP 20-20 + 50 kg ha^{-1} zeolite, V7 – 75 kg ha^{-1} NP 20-20 + 25 kg ha^{-1} zeolite, V8 – 25 kg ha^{-1} NP 20-20 + 75 kg ha^{-1} zeolite.

In order to determine soil microbiological community profile we used the MicroRespTM method (Campbell C.D. *et al*, 2003). Functional groups of microorganism were analyzed as the quantity of CO2 exported in atmosphere due to substrates decomposition. Microresp detection plates allow the evaluation of the dynamics for the

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entire microbial community, maintaining the testing conditions to the same level.

RESULTS AND DISCUSSIONS

The high potential of soybean plants to form associations with symbiotic nitrogen fixing bacteria induces strong changes in the pattern of microbial community response to fertilizers (*table 1*). Typical soil microbial community present in soybean plant (V1) is dominated by α-Ketoglutaric acid, a group proliferating due to higher exports of organic matter in the soil during the vegetation period. Codominant of this group are decomposers of D-fructose, L-malic acid and citric acid.

The basal respiration (distilled water) is maintained around the value registered in unfertilized vaiant (V1), which indicates that the size of the microbial community in soil is equal for the majority of fertilization variants (*table 1*). The lowest values were recorded in variants fertilized with ratios of 1:3 zeolite:NP 20:20 (V7 and V8), fertilization in these proportions inducing competition among microorganisms from groups located on the same feeding niche – resulting an inhibition of their proliferation.

The fertilization with urea (V2) stimulate the formation of a microbial community similar to control, with slight increases and decreases in the size of the population that metabolize D-fructose and L-malic acid (*table 1*). Fluctuations are due the supply of mineral nitrogen in soil, but fall within a normal range of variation. Instead, there are visible reductions of 2-15% of N-acetyl, γ-aminobutyric acid, D-trehalose, D-galactose, L-cysteine and L-arabinose decomposers.

Zeolite applied unilaterally (V3) creates the potential for installation of acid α-KetoGlutaric as dominant community, co-dominated by citric acid decomposeres (*table 1*). Alongside these groups, also proliferate decomposers of L-malic acid. In contrast, zeolite maintains community structure of L-cysteine and D-galactose at a level identical to typical community (V1). The balanced combination of urea with zeolite induce the proliferation of microorganisms that use L-arabinose, D-fructose, D-galactoseand some of the acids, in order to promote growth of plants. Community formed by fertilization has a profile similar to that observed at the control (V1), but the trend of citric acid group is similar to the one created by zeolite (V3). α-KetoGlutaric acid group own a clear dominance in the community formed on the balanced fertilization with urea and zeolite (V4), which indicates a strong circuit of organic matter in soils and a specialized transformation of synthetic substances. However, the segment described by the decomposition of L-lysine and L-alanine reduced greatly due to this type of fertilizer. The reduction of zeolite to only 30 kg ha^{-1} in combination with 70 kg ha^{-1} urea (V5) causes strong changes in the dominance of microbial community, citric acid group having a size similar to that of α-Ketoglutaric acid. The level of zeolite is sufficient to preserve the size of the group L-cysteine and D-galactose to values close to those of the control (V1).

Table 1

Dynamics of functional microbial groups in response to fertilization (μg CO_2-C g^{-1} h^{-1})

Substrate	V1	V2	V3	V4	V5	V6	V7	V8
Distiled water	0.75	0.81	0.73	0.74	0.71	0.82	0.43	0.52
N-acetyl-glucosamine	1.51	1.29	1.22	1.45	0.95	1.11	0.65	0.94
L-arginine	0.72	1.09	0.78	0.81	1.02	0.76	0.17	0.73
Acid γ-aminobutyric	1.03	0.95	0.84	1.12	0.98	0.98	0.52	0.65
L-lysine	0.84	0.90	0.83	0.58	1.01	0.93	0.59	0.86
L-alanine	1.34	1.40	1.17	1.10	1.06	1.06	0.72	0.79
L-cisteine	1.01	0.99	1.02	0.98	1.02	1.02	0.81	0.58
D-trehalose	2.01	1.74	1.49	1.97	1.50	1.58	0.73	1.27
D-galactose	1.67	1.56	1.67	1.92	1.67	1.71	1.04	1.55
L-arabinose	1.83	1.79	1.78	1.89	1.78	1.40	1.16	1.67
D-fructose	2.45	2.27	2.03	2.78	2.13	2.36	1.30	1.85
D-glucose	1.81	2.11	2.34	2.44	2.06	2.46	1.45	1.98
Citric acid	2.71	2.51	3.76	3.11	4.18	3.86	1.64	3.08
L-malic acid	2.16	2.36	2.43	2.57	2.42	2.78	3.75	1.83
Oxalic acid	1.36	1.49	1.46	1.59	1.67	1.45	2.45	1.30
α-ketoglutaric acid	4.16	4.27	4.33	5.28	4.29	4.51	2.66	2.79

Zeolite applied in quantities equal to NP fertilizers 20:20 (V6) acts synergistically (*table 1*) for the formation of microbial communities almost identical to that observed at the unilateral application of zeolite (V3). Zeolite acts as a buffer to reduce the distorting effect of mineral fertilizers on soil microbial dynamics, maintaining decreases in microbial community within narrow limits.

The increase of NP 20:20 dose to 75 kg ha^{-1} in the recipe fertilization (V7), while reducing zeolite only 25 kg ha^{-1}, determines a strong drift of the dominant groups in the microbial community (*table 1*). The microorganisms that decompose L-malic acid are group with the highest share in the ecosystem, co-dominated by oxalic acid and α-Ketoglutaric acid. Compared to control community (V1) a strong decrease of the group citric acid is observed at a level of 60% from its potential expansion.

By increasing zeolite to 75 kg ha^{-1} (V8) in association with only 25 kg ha^{-1} NP 20:20, there is a slight improvement in the microbial community (*table 1*). The group specialized in citric acid is the dominant group in the community, with D-fructose α-KetoGlutaric acid as co-dominants. Overall reduction in other populations is maintained in the range 5-40%. The phenomena observed in fertilized variants with unbalanced zeolite and NP 20:20 report (V7 and V8) indicate the low potential of use for this fertilizer on soybean crop, strongly disturbing the functional microbial community and affecting ecosystem stability.

The sensitivity of microbial communities to disturbances created by fertilization recipes in the experimental field, induce extreme fluctuations of oxalic acid and L-arginine groups. Removing these two groups from the analyse of microbial drift induced by fertilization leads to a large-scale image of the stability profile of microbial communities (*figure 1*).

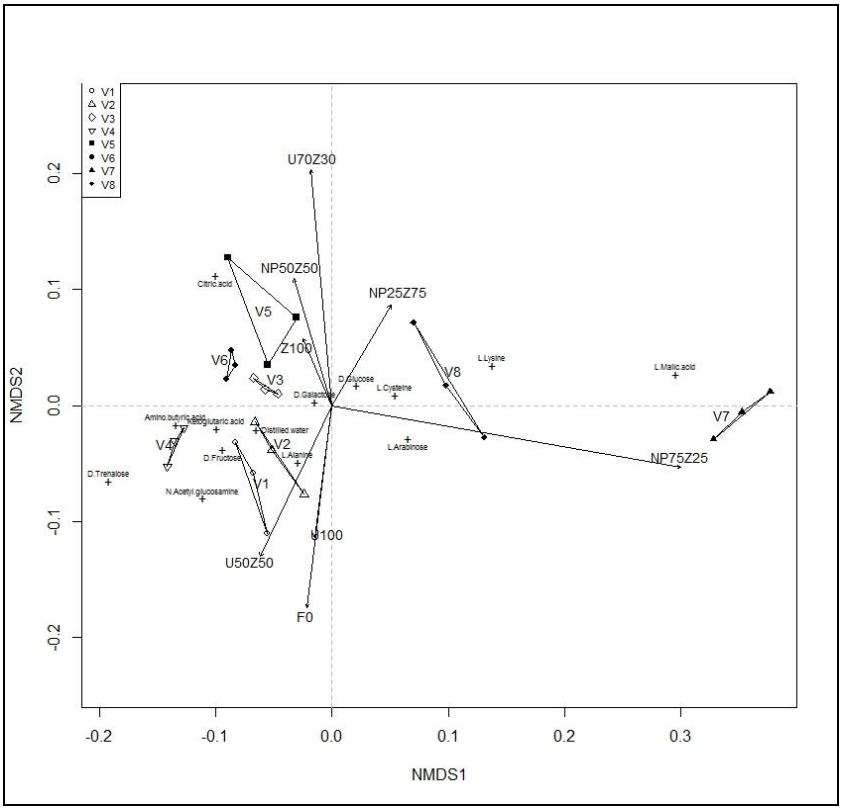

Figure 1 **Functional microbial communities determined by fertilization**

Fertilization is responsible for the oscillations of functional groups at a rate of 90.08%, Axis 1 - 89.52%, Axis 2 - 0.56% (*figure 1, table 2).* Microbial community in soil cultivated with soybeans is typical for control variant (V1, $p < 0.05$), significant depreciation is observed in the application of doses of 70 kg ha^{-1} urea in combination with 30 kg ha^{-1} zeolite (V5, $p < 0.01$) and at 75 kg ha^{-1} NP 20:20 in combination with 25 kg ha^{-1} zeolite (V7, $p < 0.01$), which supports establishing the tolerance limits for microbial community in terms of fertilization.

Fertilization with doses of 75 kg ha^{-1} NP 20:20 (V7) has a strong destabilizing character on the size and functionality of microbial communities in soil cultivated with soybean (*figure 1*), even in the presence of an additional 25 kg ha^{-1} zeolite as a buffer. Decomposition of L-malic acid, L-cysteine and L-lysine are sensitive to fertilization gradients, these groups having an

important role in highlighting the restrictive effect of mineral fertilizer dose. At doses of kg ha^{-1} zeolite (V8) associated with a 25 kg ha^{-1} NP 20:20, the disruptive potential of this recipe is visible, stimulating the overlap of antagonistic groups in the same feeding niches. This type of fertilizer produces the sensitivity for the decomposition of L-arabinose, reducing the expression potential of this group throughout the community.

The variations in specific microbial growth (*figure 1*) are reduced to equal doses of zeolite and urea (V4), NP 20:20 and zeolite (V6) and the unilateral application of 100 kg ha^{-1} zeolite (V3). Fertilization with 100 kg ha^{-1} urea (V2) increases the size of the entire microbial communities (distilled water) and produces a change in community functionality to a decomposition of L-alanine. Heterogeneous group of N-acetyl-glucosamine and D-trehalose decomposers prefer sites without fertilization (V1). The application of balanced fertilization with urea and zeolite (V4) increases the overall size of group γ-aminobutyric acid and α-Ketoglutaric acid and maintain these groups at high levels in the ecosystem.

Table 2

The importance of fertilization in the dynamics of microbial communities

	NMDS1	NMDS2	r^2	Pr(>r)
V1	-0.123	-0.992	0.270	0.049 *
V2	-0.131	-0.991	0.118	0.252
V3	-0.394	0.919	0.035	0.755
V4	-0.431	-0.903	0.181	0.099 .
V5	-0.0879	0.996	0.365	0.006 **
V6	-0.286	0.958	0.115	0.251
V7	0.985	-0.174	0.818	0.002 **
V8	0.504	0.864	0.088	0.355
*Signif . codes: 0 '***' 0.001 '**' 0. 01 '*' 0.05 '.' 0.1 ' ' 1*				

The absence of fertilization (V1) induces reduced depreciation in the size of oxalic acid and L-malic acid decomposers, but without a strong effect on the total amount of microbial communities (*table 2*). Instead, in the absence of external inputs (V1) the N-acetyl-glucosamine and D-trehalose decomposition is significantly enhanced. On the other hand, urea applied unilaterally (V2) significantly increases the active degradation of L-alanine and L-arginine, with a reduced influence on other microbial functional groups.

Table 3

Correlation of fertilization with microbial functional groups

Substrate	V1	V2	V3	V4	V5	V6	V7	V8
N.Acetyl.glucosamine	0.49*	0.20	0.10	0.41*	-0.25	-0.04	-0.66***	-0.26
Oxalic.acid	-0.24	-0.11	-0.14	-0.01	0.08	-0.15	0.89***	-0.31
D.Trehalose	0.46*	0.20	-0.04	0.43*	-0.04	0.04	-0.78***	-0.26
Distilled.water	0.15	0.32	0.12	0.13	0.05	0.35	-0.68***	-0.44*
Amino.butyric.acid	0.26	0.11	-0.07	0.43*	0.18	0.17	-0.66***	-0.42*
Ketoglutaric.acid	0.05	0.10	0.13	0.54**	0.11	0.21	-0.60**	-0.55**
L.Lysine	0.05	0.20	0.03	-0.59**	0.48*	0.28	-0.56**	0.10
L.Malic.acid	-0.23	-0.05	0.01	0.14	0.01	0.33	0.30	-0.52**
L.Cysteine	0.16	0.13	0.18	0.11	0.18	0.17	-0.24	-0.69***
D.Fructose	0.27	0.11	-0.10	0.56**	-0.02	0.19	-0.74***	-0.26
D.Galactose	0.10	-0.06	0.12	0.49*	0.10	0.16	-0.84***	-0.07
D.Glucose	-0.29	0.03	0.28	0.38	-0.02	0.40	-0.67***	-0.11
L.Alanine	0.37	0.45*	0.13	0.03	-0.03	-0.03	-0.51*	-0.42*
L.Arabinose	0.21	0.16	0.15	0.28	0.14	-0.33	-0.62**	0.01
L.Arginine	-0.05	0.47*	0.02	0.07	0.37	0.00	-0.83***	-0.05
Citric.acid	-0.17	-0.26	0.29	0.00	0.47*	0.33	-0.64***	-0.01
*p<0.05 * / p<0.01 ** / p<0.001***								

Zeolite in quantities of 100 kg ha^{-1} (V3) is the most consistent method of stabilizing microbial communities, with global insignificant disturbance influences over the entire microbial community (*table 2*). The potential of zeolite to restore soil natural fertility is also highlighted, by balancing metabolic processes and microbial interactions between the functional groups.

At the associated application of zeolite with urea (V4) the entire segment of N-acetyl-glucosamine, D-trehalose, γ-aminobutyric acid, α-Ketoglutaric acid and D-galactose decomposition is significantly stimulated, but the reduction of L-lysine decomposition is visible, which indicates a restriction of ecological niche (*table 3*). Zeolite dose of 30 kg ha^{-1} in combination with urea (V5) restricts the growth rate of microbial populations, maintaining a level similar to that of typical community (V1). The only functional group that proliferates significantly at the application of the fertilizer recipes is responsible for citric acid decomposition.

Fertilization with 75 kg ha^{-1} NP 20:20 (V7) associated with only 25 kg ha^{-1} zeolite significantly reduces the vast majority of microbial functional groups (*table 3*), which indicates the restriction of the contact interface between soybean plants and microbial communities. The only group that proliferated under the conditions of this type of fertilizer is the citric acid, due to the existence of high levels of nitrogen in soil and the decomposition of the zeolite. Reducing the amount of nitrogen to 25 kg ha^{-1} (V8) along with an increase of zeolite induces an improvement in the disturbance caused by the nitrogen and phosphorus that are directly accessible to plants, but a reduction of the entire microbial community (distilled water) is still observed. In this particular case the reduction of α-Ketoglutaric acid, γ-aminobutyric acid, L-alanine, L-malic acid and L-cysteine decomposers is visible, so the direction of microbial community is focused on the degradation of easily accessible resource.

The stability induced by fertilization of all the experiment reveal variants with high efficiency in order to restore the natural fertility of soil. There are also high disruptive impact variants (*figure 2*). The fertilization with urea at 100 kg ha^{-1} (V2) induces a community similar to that observed in the control variant (V1). This phenomenon is caused by the capacity of bacterial symbionts in the roots of soybean to fix nitrogen, which causes the parity between the two variants in the amount of nitrogen in soil.

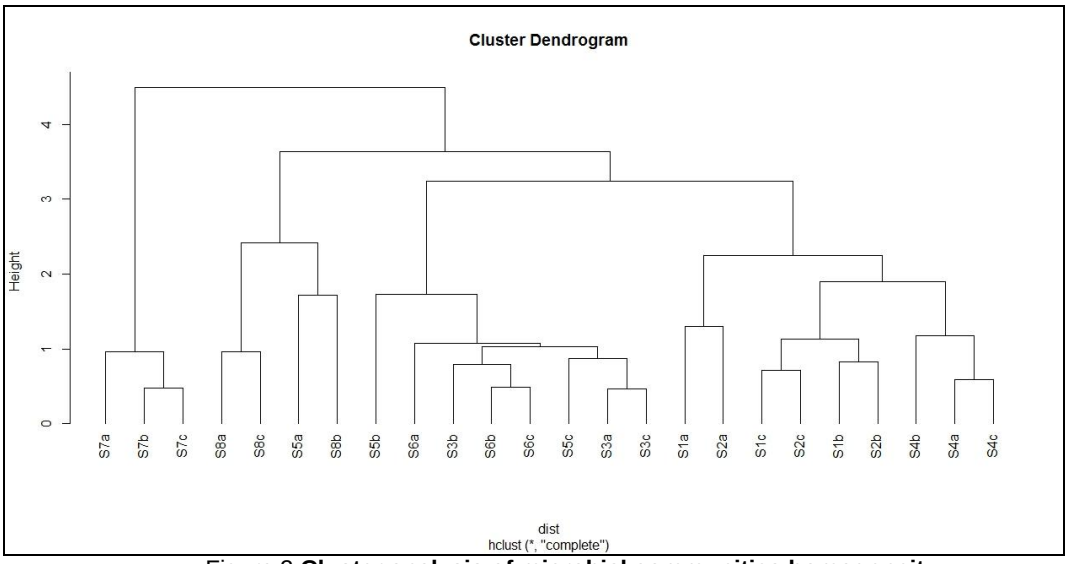

Figure 2 **Cluster analysis of microbial communities homogeneity**
(*Legend: S=soybean, no. 1-8 = number of variant, a-c = replications*)

A typical community similar to that observed in the control group (V1) is observed in the variant fertilized with zeolite and urea in quantities of 50 + 50 kg ha^{-1} (V4), but with high stability over time and lower local oscillation. This promotes the concept of balanced fertilization for soybean crop in order to maintain the functional microbial communities in the rhizosphere. Zeolite applied unilaterally (V3) produce strong local variations in microbial community by activating functional groups with antagonistic processes. The application of NP 20:20 and zeolite (V7) produce a strong depreciation of the microbial community. Compared to control community, this variant induces the synthesis of a community with a high degree of specialization in use of nitrogen and phosphorus, but with high stability and low spontaneous variations.

CONCLUSIONS

Microorganisms from soybean rhizosphere have a strong reaction to associated fertilization, zeolite acting as a buffer for disturbances caused by the mineral component of fertilizer recipes.

Decomposers of α-Ketoglutaric acid are the dominant group in the rhizosphere of soybean fertilized with urea and zeolite.

At low levels of zeolite appears a double dominance of α-KetoGlutaric acid and citric acid decomposers.

Microbial functional drifts produced by high ammount of NP 20:20 create the potential of citric acid decomposers to become dominant in the rhizosphere.

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