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SOIL MICROBIOTA UNDER THE CONDITIONS OF AN OPEN FIELD EXPERIMENT OF FERTILIZING SOIL BY ENERGY WILLOW

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The aim of the work was to study the soil agrochemical indices, soil microbiocoenosis, in case of growing of energy cultures and based on the mineralization coefficient, to make a conclusion on the speed of mineralization processes in the soils under study. In conditions of continuous field experiment (2011–2016), the dynamics of soil microbial associations was studied for willow (*Salix triandra* × *Salix viminalis* 'Inger') cultivation with application of experimental fertilizers of different types. In the research fertilizers there were used: sulfuric urea, municipal biocompost, municipal sewage sludge compost, rhyolite tuff and willow ash. The soil microbiotic communities analysis was conducted by the method of serial dilutions of soil suspension with the use of differentially diagnostic nutrient media: meat-peptone agar, starch-ammonium agar, Ashby medium, potato agar, Czapek Dox medium, starvation agar, Ploskirev medium. The direction of the microbiological processes in the soils was determined.

According to the results, it was established that the most promising for the purpose of improving the metabilic activity of the soil (in the growth of energy willow) is a municipal sewage sludge compost and a municipal biocompost. In case of the use of municipal sewage sludge compost, the number of intestinal bacteria, ammonifiers, micromycetes and actinomycetes was doubled as compared with the control. In case of the use of municipal biocompost, the levels of microscopic fungi and cellulolytic bacteria doubled, and those of intestinal bacteria and pedotrophs tripled as compared with the control. While calculating the mineralization/immobilization index, it was shown that the most significant deviation from the control plot was found in the rhyolite tuff treated soil — a decrease by 6 times, and in case of willow ash by 2.3 times, which proved the inhibition of mineralization of the organic substances in the soil.

Key words: energy willow, organic and inorganic soil additives, soil microbiocoenosis, mineralization coefficient.

To date there have been known about 20 species of fast-growing plants that may be grown to obtain high amounts of plant biomass — e.g. eucalypt, poplar, willow, silver grass, giant reed, and others. The harvested biomass is used for the production of heat and electric energy, and may be used as primary product to produce such biofuel in the form of granules and bricks [1–6].

In the EU countries (Sweden, Denmark, United Kingdom), the use of energy cultures is especially popular. In Ukraine, plantations of energy willow are grown in Kyiv, Donetsk, Ivano-Frankivsk, Volyn, Lviv, Ternopil and Rivne oblasts (regions). Transcarpathia (Zakarpatska oblast) also has a more than ten-year-long experience of growing energy willow (*Salix* sp.).

This culture is used not only as an energy source but with the aim of bio- or phytoremediation. Willow may efficiently grow on the soils with heightened level of lead and cadmium [7], for instance along the roads or near industrial facilities. Its wood may be used as biofuel [3]. At the same time, willow is a very fast-growing plant, which fact allows it to be used for phytoremediation. What is more, various willow species are considered as promising plants for Ukraine's urban landscaping [8]. Earlier we have analyzed changes in the composition of the soil microbial coenosis occurring when growing energy willow, compared to the soils of the meadow plot [9, 10]. There have been many reports claiming that energy willow rapidly exhausts the stocks of soil nutrients [11], which is why the aim of our research has been to study the soil agrochemical indices and soil microbiocoenosis, in case of growing of energy cultures; to compare the obtained data with the control (a meadow ecosystem); and based on the mineralization coefficient, to make a conclusion on the speed of mineralization processes in the soils under study.

Materials and Methods

Open-field small-plot long-term experiment was set up with energy willow (*Salix triandra*×*Salix viminalis* cv. Inger; license holder: Lantmännen Agroenergi AB, Sweden; Hungarian distributor: Holland-Alma Ltd., Piricse) during April of 2011. Research area is located in parallel to Westsik street in Nyíregyháza city (Hungary) in the experimental field of Research Institute of Nyíregyháza — University of Debrecen, Centre of Agricultural Sciences. The basic characteristics of the uncontaminated brown forest soil were the following at 0–25 cm layer: loamy sand texture; pH H_2O 8,10; pH KCl 7.52, $CaCO_3$ (m/m%): 4,80; total salt content (m/m%): < 0.02; humus 1.51%; CEC 10.4 cmolc/kg; NH_4-N (mg/kg): 5.68; NO_3-N (mg/kg): 6.37; P — 713, K — 5653, Ca — 21773, Mg — 5471, Cu — 12.7; Mn — 653, Zn — 44.3; As — 38.3; Cd — 0.11; Pb — 13.6 mg/kg in HNO_3–H_2O_2 extract. The experiment was set up during 2011 on random-block design with 40 small plots (3800 m^2), 10 various treatments and with 4 replications. In one 27 m^2 plot 40 willow bushes are grown. During April and May of 2016 the main soil treatments were the following:

• *Control* (without any fertilization since 2011).

• *Sulfuric urea* (SU) — 100 kg/ha dry weight with 46% nitrogen (producer Nitrogénművek Vegyipari Co., Pétfürdő, Hungary); applied as top-dressing.

• *Municipal biocompost* (MBC) — 20 t/ha wet weight with 75–76% dry matter (producer Térségi Hulladék-Gazdálkodási Ltd., Nyíregyháza, Hungary); applied also with the same dose during the spring of 2011 and 2013.

• *Municipal sewage sludge compost* (MSSC) — 15 t/ha wet weight with 48–56% dry matter (producer Nyírségvíz Ltd., Nyíregyháza, Hungary); applied also with the same dose during the spring of 2011 and 2013.

• *Rhyolite tuff* (RT) — 30 t/ha wet weight with 18% moisture content (producer Colas-Északkő Bányászati Ltd., Tarcal, Hungary); applied with this dose only during the spring of 2011 and 2013 (not in 2016).

Willow ash (WA) — 300 kg/ha dry weight with 1% moisture content (produced at the University of Nyíregyháza with burning of willow shoots without leaves). 600 kg/ha WA was applied to the soil during 2011 and 2013, respectively.

Above fertilizers and additives were immediately rotated to upper 0–25 cm layer of the soil. The basic physical and chemical characteristics (plant nutrient content) of municipal biocompost, municipal sewage sludge compost, rhyolite tuff and willow ash were described in our previous studies [12–14].

The soil sampling for study soil microbiota was done by August 8, 2016. The upper 5 cm layer of the soil was removed, and then sampling was performed with the help of a drill rod from 10–15 cm soil depth. Mixed average samples were taken combining 5 soil subsamples per plot, which were taken from the neighbourhood of willow plants, located in centre of the given experimental plot. From every treatment 2 independent average samples were taken from 2 parallel plots, receiving the same treatments. All soil samples were put in snap-lockable plastic bags, and were transported immediately to laboratory in Uzhgorod in a cooler box.

The soil microbial coenosis analysis was conducted with the use of differentially diagnostic nutrient media by the method of serial dilutions of soil suspension. The *ammonifying bacteria* were propagated on meat-peptone agar (MPA); the *actinomycetes* and *mycobacteria* — on starch-ammonium agar (SAA); the *oligotrophs* — on Ashby medium; the *myxobacteria* — on potato agar; the *micromycetes* — on Czapek Dox medium; the *oligonitrophils* — on starvation agar; the *Azotobacter* — on Ashby medium using the method of soil lumps fouling; the *enteric bacteria* — on Ploskirev medium [15]. The results were evaluated by the number of colony-forming units per 1 g of absolutely dry soil (CFU/g) [15].

The soil was selected from each plot of the experiment in two replicates (parallel treatments). All microbiological tests were conducted with 3 replications.

The direction of the microbiological processes in the soils was determined after [16]. The mineralization/immobilization index (MII) was calculated as the ratio of the number of microorganisms grown on starch-and-ammonia agar and meat-infusion agar, correspondingly. The pedotrophic index (PI) was calculated as the ration of microorganisms grown on meat-infusion agar and soil agar.

Statistical analysis of experimental data was conducted with MS Excel 10.0 software using analysis of a variance (ANOVA) followed by treatment comparison using Tukey's test. The Tukey Test is a post-hoc test based on the studentized range distribution. The test compares all possible pairs of means. The Tukey test is invoked when you need to determine if the interaction among three or more variables is mutually statistically significant. Honestly significant difference is shown when the pairwise difference between two means exceeds the value. Hereinafter the letters "a" etc. indicate statistically significant differences.

Results and Discussion

In Table are demonstrated the regularities in distribution of certain physiological groups of microbial coenosis in conditions of growing energy plants.

Ammonifiers. A statistically reliable growth of ammonifiers was observed in the version of the experiment with the use of municipal sewage sludge compost.

Enterobacter. The number of intestinal bacteria was observed to grow, with the use of top-dressing (SU) their number doubled; with the use of municipal biocompost (MBC) it tripled; and with the use of municipal sewage sludge compost (MSSC) it grew by 2.5 times. The lower number of intestinal bacteria was found in plots to be growing, if rhyolite tuff (RT) and willow ash (WA) was applied.

Micromycetes. The number of mictomycetes was observed to be growing if RT, MBC, or — to the utmost — MSSC were used.

Actinomycetes. The number of bacteria using mineral forms of nitrogen was growing if MSSC, or MBC were used. A considerable decrease in the number of bacteria using mineral forms of nitrogen was found when RT was used. The number of these bacteria in plots to be growing with the use SU and WA did not differ from the control plot.

Pedotrophs. The number of pedotrophs was observed to be growing in the soil of plot to be growing if SU top-dressing, and if MBC and RT were used.

Myxobacteria. No significant myxobacteria changes were found in the soils of the experimental plots, however it is worth noting their lowering only in the soil of to be growing if WA was applied.

Oligonitrophils. The number of oligonitrophils was observed to be decreasing with the use of MSSC, WA, and RT.

Celulosolytic organisms. The number of cellulosolytic microorganisms was found to decline in case of the use of MSSC, and RT.

Scientific literature is aware of such regularities. The stimulating activity of municipal sewage upon the content of most bacterial and fungal groups was also proved experimentally by the example of grey-brown podzolic soils, during both the first and the second year of the experiment [17]. When secondary-treated municipal sewage was added to the soil with willows, the microbial biomass, microbial breathing and fermentative activity were observed to grow [18].

In case of the use of MBC, rise in the levels of intestinal bacteria, microscopic fungi, actinomycetes, pedotrophs and cellulosolytic bacteria was found (Table).

The calculated coefficients and indices reflected the elements of the morpho-functional structure of microbial coenoses, and revealed the direction of the soil processes. The *mineralization/immobilization index* (MII) shows the intensity of the processes of mineralization and assimilation of nitrogen compounds, and characterizes the level of intensity of mobilization processes in soil. The *pedotrophic index* characterizes the level of assimilation of soil organic substances by microorganisms and thus the functionality of the structure of the soil microbial coenosis. Low pedotrophic indices were evidence of the inhibition of mineralization of the organic substances. Increase of the pedotrophic index testified to the growing transformation intensity of the organic substances [19].

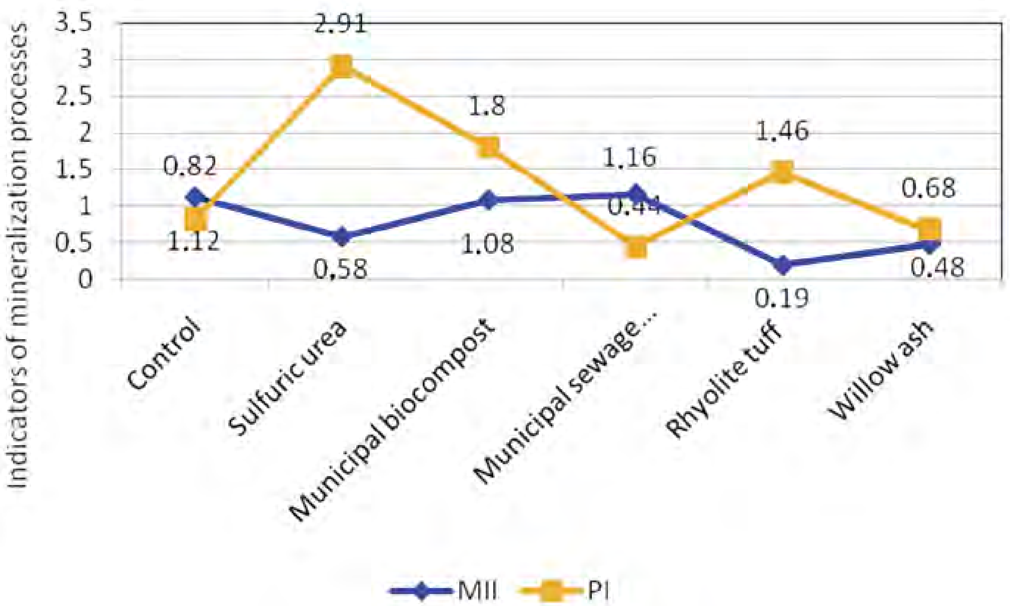
In case of well-balanced ratio between the processes of synthesis and decomposition of organic substances, the values of mineralization indices approximated one. These were the trends observed in case of the control plot. In that case, the mineralization/immobilization index equaled to 1.12 (Figure).

Similar values were observed in the treatments MBC and MSSC (1.08 and 1.16, respectively). The approximation of the given values to the control values coincided with the heightened number of actinomycetes in the soils. Halving of the mineralization/immobilization index was observed in the soils with SU and WA added. In case of addition of

Soil microbiocoenosis after soil application of various additives and an artificial fertilizer as a top-dressing*

Treatments	Group of microorganisms, CFU/g of soil $\times 10^6$, $M \pm \sigma$							
	Ammonifiers	Enterobacter	Micromycetes	Actinomycetes Mycobacteria	Pedotrophs	Myxobacteria	Oligonitrophils	Celulosolytic microorganisms
Control	2.0±0.9 a	0.5±0.1 b	0.4±0.1 b	2.2±1.2 b	1.6±0.2 a	0.4±0.1 c	3.2±0.4 c	1.2±0.2 d
SU	2.8±1.3 a	1.1±0.1 c	0.2±0.1 a	1.6±0.1 b	8.0±1.0 d	0.3±0.1 ab	2.3±0.1 b	1.5±0.1 e
MBC	2.7±0.5 a	1.5±0.3 cd	0.9±0.1 d	2.9±0.6 bc	5.1±0.9 c	0.2±0.1 a	3.5±0.2 c	2.0±0.5 f
MSSC	4.5±1.2 ab	1.2±0.1 c	1.7±0.1 f	5.2±0.9 d	2.0±0.6 a	0.3±0.1 c	1.2±0.2 a	0.3±0.1 a
RT	3.0±1.7 a	0.3±0.1 a	1.2±0.1 e	0.6±0.2 a	4.4±0.6 c	0.4±0.1 cd	1.4±0.6 a	0.6±0.1 b
WA	3.0±0.9 a	0.4±0.1 a	0.5±0.1 c	1.4±0.1 b	2.0±0.2 ab	0.2±0.1 a	1.0±0.4 a	0.9±0.1 c

**Hereinafter:* open-field long-term experiment. August, 2016. Nyíregyháza, Hungary.
Abbreviations: SU — sulfuric urea; MBC — municipal biocompost; MSSC — municipal sewage sludge compost; rhyolite tuff — RT; willow ash — WA.
Hereinafter: data are means of 3 replications in two parallel plots ANOVA Tukey's test. The letters a–d indicate statistically significant differences in the level of microorganisms ($P < 0,05$); $M \pm m$; control — without any fertilization.



Impact of various additives and an artificial fertilizer as a top-dressing on the intensity of mobilization processes in soil, when growing energy willow

RT, the given index was observed to go down considerably (0.19).

The pedotrophic index in the control equaled to 0.82. It went up in variants SU, MBC and RT. With MBC and WA added, the value of the pedotrophic index was observed to halve to 0.44 and 0.48, respectively.

Based on the detailed analysis of the mineralization indices, we observed that the variant with addition of MBC was the closest to the control. The obtained results proved concordant with other authors' data. Introduction of compost into soil with energy willow plantations was observed to change the direction of environmental links, and increase the integrity and resistance of the "soil — microorganisms — plant" system [20].

Thus, the most indicative changes in the soil microbiota against the control plot were found in case of the use of municipal sewage sludge compost: rise in the number of intestinal bacteria, ammonifiers, micromycetes and actinomycetes, and fall in the number of oligonitrophils and cellulosolytic microorganisms.

While calculating the mineralization/immobilization index, it was shown that the most significant deviation from the control plot was found in variant of rhyolite tuff — a decrease by 6 times, and in case of willow ash in 2.3 times, which proves the inhibition of mineralization of the organic substances in the soil.

In all treatments of the experiment, the number of free-living nitrogen-fixing microorganisms (*Azotobacter*) was found to be equal to 100% .

Calculation of the pedotrophic index showed growth of humus formation speed in the soils of sulfuric urea top-dressing and municipal biocompost, while in plot of municipal sewage sludge compost the pedotrophic index was found to be decreasing significantly.

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МІКРОБІОТА ҐРУНТУ В УМОВАХ ВІДКРИТОГО ПОЛЬОВОГО ЕКСПЕРИМЕНТУ З УДОБРЮВАННЯ ҐРУНТУ ЕНЕРГЕТИЧНОЮ ВЕРБОЮ

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Метою роботи було вивчити ґрунтові агрохімічні показники, мікробіоценоз ґрунту в разі вирощування енергетичних культур та на основі коефіцієнта мінералізації зробити висновок про швидкість процесів мінералізації на досліджуваних ґрунтах. В умовах польового довготривалого експерименту (2011–2016 рр.) досліджено динаміку мікробних асоціацій ґрунту під час вирощування верби (*Salix triandra*× *Salix viminalis* 'Inger') за внесення експериментальних добрив різних видів. У дослідженнях використано такі види добрив: сірчану

МИКРОБИОТА ПОЧВЫ В УСЛОВИЯХ ОТКРЫТОГО ПОЛЕВОГО ЭКСПЕРИМЕНТА ПО УДОБРЕНИЮ ГРУНТА ЭНЕРГЕТИЧЕСКОЙ ИВОЙ

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Целью работы было изучить грунтовые агрохимические показатели, микробиоценоз почвы, при выращивании энергетических культур и на основе коэффициента минерализации сделать вывод о скорости процессов минерализации на исследуемых почвах.

В условиях длительного полевого эксперимента (2011–2016 гг.) исследована динамика микробных асоциаций почвы при выращивании ивы (*Salix triandra*×*Salix viminalis* «Inger») и внесении экспериментальных удобрений различных видов. В исследованиях использованы следующие виды удобрений:

сечовину, муніципальний намул, муніципальний біокомпост, ріолітовий туф і золу верби. Ґрунтовий мікробіоценоз визначали методом посіву серійних розведень ґрунтової суспензії з використанням диференційно-діагностичних живильних середовищ: м'ясопептонний агар, крохмале-аміачний агар, середовище Ешбі, картопляний агар, середовище Чапека–Докса, голодний агар, середовище Плоскірєва. Визначали інтенсивність мікробіологічних процесів у ґрунтах.

За результатами досліджень встановлено, що найбільш перспективними з метою поліпшення мікробного угруповання ґрунту (в разі вирощування енергетичної верби) є муніципальний намул та муніципальний біокомпост. За використання муніципального намулу спостерігали збільшення кількості ентеробактерій, амоніфікаторів, мікроміцетів та актиноміцетів удвічі порівняно з контролем. У разі застосування муніципального біокомпосту виявлено підвищення рівня мікроскопічних грибів та целюлозолітичних бактерій — удвічі, а кишкових бактерій та педотрофів — утричі порівняно з контролем. За обчислення індексу мінералізації/іммобілізації встановили, що найбільше відхилення від контрольного варіанта виявлено в ґрунті, обробленому ріолітовим туфом, — зменшення у 6 разів, у разі застосування золи верби — в 2,3 раза, що свідчить про гальмування мінералізації органічних речовин у ґрунті.

Ключові слова: енергетична верба, органічні та неорганічні добрива, мікробіоценоз ґрунту, коефіцієнт мінералізації.

серную мочевину, муниципальный ил, муниципальный биокомпост, риолитовий туф и зола ивы. Почвенный микробоценоз определяли методом посева серийных разведений почвенной суспензии с использованием дифференциально-диагностических питательных сред: мясопептонный агар, крахмало-аммиачный агар, среда Эшби, картофельный агар, среда Чапека–Докса, голодный агар, среда Плоскирева. Определяли интенсивность микробиологических процессов в почвах.

По результатам проведенных исследований установлено, что наиболее перспективными с целью улучшения микробной группировки почвы (при выращивании энергетической ивы) являются муниципальный ил и муниципальный биокомпост. В случае использования муниципального ила выявлено увеличение количества энтеробактерий, аммонификаторов, микромицетов и актиномицетов вдвое по сравнению с контролем. В случае использования муниципального биокомпоста выявлено повышение уровня микроскопических грибков и целлюлозолитических бактерий — вдвое, а кишечных бактерий и педотрофов — втрое по сравнению с контролем.

При вычислении индекса минерализации/иммобилизации установлено, что наибольшее отклонение от контрольного варианта обнаружено в почве, обработанной риолитовым туфом, — уменьшение в 6 раз, в случае применения золы ивы — в 2,3 раза, что свидетельствует о торможении минерализации органических веществ в почве.

Ключевые слова: энергетическая ива, органические и неорганические удобрения, микробиоценоз почвы, коэффициент минерализации.

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