

# **PhD THESIS**

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**SZENT ISTVÁN UNIVERSITY**

**THE EFFECT OF DIFFERENT MANAGEMENT ON THE FUNCTIONAL DIVERSITY  
OF SOIL MICROBIAL COMMUNITIES**

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**GÖDÖLLŐ**

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## 1. BACKGROUND AND OBJECTIVES

Soil microbe community is a key factor in the soil agro-ecosystem as it affects quality and quantity of food production (WILLIAMSON et al. 2011, van LEEUWEN et al. 2017). In the development of agro-ecosystems the choice of the appropriate area-specific farming method is a very important factor as it contributes to the maintenance of soil health and soil fertility (PETRIC et al. 2011).

Conventional farming focuses on productivity and quantity, while organic farming aims to ensure the quality of the food produced. Intensive tillage systems are characterized by the mechanized and specialized soil usage, significant use of fertilizers, pesticides and feed. Therefore agro-ecosystems are heavily polluted with these chemicals.

Scientific data shows that organic farming has a positive effect on soil quality and fertility (for example: soil nutrient degradation, diversity of endemic-symbiotic soil microbial populations, enzyme activity) and for this it is receiving more attention today as a sustainable agricultural system.

In the past few years the number of areas under organic cultivation has been growing dynamically in Hungary, however, it is dwarfed by conventional cultivation both in terms of area of production and proportion of producers. Therefore it is appropriate to provide a related scientific research background and to develop research-based practices (REEVE et al. 2016, GAZDAG et al. 2018).

There are no standard methods for determining soil quality and soil health. In soil quality assessment the biomass, genetic diversity, activity and impact of soil microorganisms on soil fertility, as well as responses to stress factors are important attributes.

Basically in Hungary there are no comparative and complex studies on soil microbial communities of different soil types and cultivation methods. Most of the available literature describes the effect of different treatments on only one type of soil, or compares several soil types, but only taking into account one microbiological characteristic. For the application of soil microbiological, physical and chemical parameters in a monitoring system a well-calibrated reference base is required for the interpretation of the results (SZILI-KOVÁCS et al. 2009).

The choice of soil biological indicator variables is a key and critical point in the assessment of soil quality, as the characteristics of the soil under study and the objectives have a major influence on it (NORTCLIFF 2002). The so-called minimum data set (MDS) (SZILI-KOVÁCS et al. 2011) is widely used in the evaluation of soil quality (SHUKLA et al. 2006, CHUN-JUAN et al. 2013).

The main goal of my PhD research was to examine the composition and functionality of soil microbe community in a complex way (with MDS approach) with classical status (main soil physics and chemical parameters) and functional tests (genetic and functional diversity, enzyme activity, respiration) on the organic and conventional cultivation of 3 soil types.

Soil bacterial populations also have long-term effects in healthy food production so research of the different types of farming (SCHLOTTER 2003, GARBISU et al. 2011, GAZDAG et al. 2019b) is crucial.

During the research I examined different soil types and farming methods with different texture (Karcag: clayey soil (organic), clayey-loam soil (conventional), Martonvásár: loamy soil

(organic), loam soil (conventional), Nyíregyháza: sandy-loam soil (organic), sandy soil (conventional)) and how they change the diversity, the relative proportions and the composition of the soil bacterial communities.

The multicriteria analysis applied to the main objectives of the PhD research consists the following components:

**1. Characterization of the soils of the experimental areas on the basis of their major physical and chemical properties**

- Physical soil type
- EC, pH<sub>H<sub>2</sub>O</sub>, nitrogen forms, humus and carbon content
- AL-soluble nutrient contents, main meso and micro element contents

**2. Functional investigation of the soils of experimental areas**

- Fluorescein-diacetate hydrolysis (FDA)
- Basal respiration (BRESP)
- Microbial biomass (SIR)
- Physiological pattern analysis at Community level (CLPP: MicroResp™)
- Colony forming units (CFU)

**3. Molecular genetic studies of soil in experimental areas**

- Methodological development of bacterial DNA isolation from soil
- PCR, optimization of nested-PCR
- DGGE optimization
- sequencing

**4. Multi-criteria complex evaluation of the above performance characteristics included in the analysis taking into account several aspects at the same time**

- One-way analysis of variance (ANOVA)
- Pearson-correlation
- Rank-correlation (SRD)

My results draws attention to the fact that the above-mentioned multicriteria analysis is essential for understanding the role of soil microbial communities of the rhizosphere and it is necessary to understand the sustainability of this management method. These statements may provide important reference data for the assessment of soil quality, soil fertility and degradation processes. Furthermore the multicriteria analysis also highlights that there may be significant differences in the sensitivity of the indicators used to characterize the microbial community of soil.

## 2. MATERIALS AND METHODS

### 2.1. Characterization of experimental fields

The main criteria for the designation of the sampling areas was the different physical characteristics of the soils originated from different farming methods (organic and conventional). Our sample plots represents three different physical soils and are located in Martonvásár, Karcag and Nyíregyháza. The soil samples originated from the rhizosphere. Soil sampling was carried out in autumn, 2011 and in spring, 2012. In the three areas I sampled an organic and a conventional parcel. Soil samples were taken from 0-20 cm soil depths on every 10 meters from a sampling area in 12 replicates. A total of 144 soil samples were collected from the three areas over the two years. A part of soil samples were homogenised, air-dried, grounded, and sieved through a 2 mm mesh for physico-chemical analysis. The remaining soil samples were stored at +4°C for up to one week for microbiological analyses and at -20°C for molecular analyses until further laboratory processing.

Based on the soil physics analysis of the samples, their particle size classification is based on the texture triangle:

- Karcag, organic management, clay soil =AO
- Karcag, conventional management clay loam soil=AVK,
- Martonvásár, organic management loam soil=VO,
- Martonvásár, conventional management loam soil=VK,
- Nyíregyháza, organic management loamy sandy soil=VHO,
- Nyíregyháza, conventional management, sandy soil=HK.

#### 2.1.1. Karcag experimental area

Soil sampling was carried out at the Research Institute of Karcag, Institutes for Agricultural Research and Educational Farm of University of Debrecen. The sampled conventional area is OMTK 17, AI. 16. parcel (5,67 ha). At the time of sampling sorghum and winter wheat were sown in 2011 and winter wheat in 2012. In the case of organic cultivated areas, samples were taken from the M1 parcel (5,67 ha). Duckweed (2011; *Melilotus*; Fabaceae) and millet (2012; *Panicum miliaceum*, Poaceae) were sampled at the site, with the following GPS coordinates: 47° 16' 47'' N, 20° 53' 12'' E. The Karcag soil is a meadow chernozem (STEFANOVITS 1972), (WRB) with a heavy clayey loam texture (FAO 2015).

#### 2.1.2. Martonvásár experimental area

In the past 20 years before the sampling neither fertilizer nor pesticide were applied in the certified organic plots of Centre of Agricultural Research, Hungarian Academy of Sciences in Martonvásár (MIKÓ et al. 2014). Green peas (2011; *Pisum sativum*; Fabaceae) and cereals (2012) were sown in the area during the soil sampling years and then cereals were sown in the autumn. The cultivated plots are located next to the organic area where spring wheat (2011; *Triticum aestivum*; Poaceae) and maize (2012; *Zea mays*; Poaceae) were sown in the year of sampling. Martonvásár soil is a clayey loam chernozem (STEFANOVITS 1972), (WRB) (FAO 2015). Both areas are 0,5-0,5 hectares with the following GPS coordinates: 47° 18' 38'' N, 18° 46' 45'' E.

### 2.1.3. Nyíregyháza experimental area

The Westsik crop rotation experiment is located on the territory of Institutes for Agricultural Research and Educational Farm of University of Debrecen. Soil samples were taken from the bottom of the hill to the top (6,70 hectares) on parcel 15 (47° 58' 42" N, 21° 40' 52" E). During the sampling years rye (*Secale cereale*, Poaceae) (2011) and rye and fluffy vetch (*Vicia villosa*, Fabaceae) (2012) were sown in the area. Organic farming area of the Research Institute of Nyíregyháza was sampled from plot 16 (14,9 ha), 47° 58' 48" N, 21° 40' 33" E, where spelt wheat (*Triticum spelta*, Poaceae) and alfalfa (*Medicago sativa*, Fabaceae) were grown in 2011, while in 2012, buckwheat (*Fagopyrum esculentum*, Polygonaceae) and peas (*Pisum sativum*, Fabaceae) were grown. The soil is humus sandy soil (STEFANOVITS 1972), (WRB) (FAO 2015).

## 2.2. Status tests

### 2.2.1. Main physical and chemical properties of soils

The physico-chemical properties of soils were examined in the main laboratory of the Institute for Soil Sciences Agricultural Chemistry, Hungarian Academy of Sciences. Water of condition was determined by the oven method (105 °C to constant weight). The texture of soils was determined by the MSZ 08-0205:1978 (BUZÁS et al. 1993) method – clay and loamy soil by pipetting, sandy soil by sifting. Total salt content was measured from the electric conductivity (EC 2.5) of soil: water (1:2.5, [w/w]) suspensions. pH<sub>(H<sub>2</sub>O)</sub> was measured according to MSZ 08-0206-2:0178.

KCl-soluble NH<sub>4</sub><sup>+</sup>-N (mg/kg)- és NO<sub>3</sub><sup>-</sup>N (mg/kg) and total N (%) contents was measured by MSZ 08-0458-80 (TYURIN 1937, BARANYAI et al. 1987). Humus content was calculated according to (%) TYURIN (1937). AL-soluble Ca (m/m%), K<sub>2</sub>O (mg/kg), Na (mg/kg), P<sub>2</sub>O<sub>5</sub> (mg/kg) contents were determined by MSZ 20135:1999 (EGNÉR et al. 1960).

Macro- and microelements (Mg, B, Cu, Fe, Mn, S, Zn) were measured by an inductively coupled plasma atomic emission spectrometer (ULTIMA 2 ICP Optical Emission Spectrometer, Jobin Yvon Technology, HORIBA France SAS, Montpellier, France) according to MSZ 21470-50:2006.

## 2.3. Functional tests

### 2.3.1. Soil microbiological studies

Ten-fold dilutions of soil suspensions were prepared in sterilized tap water (SZEGI 1979, BORSODI 2018). One hundred microliters of each dilution was used for the inoculation. Three plates were used for each dilution (KÖDÖBÖCZ et al. 2013). *Bacillus* sp. and spore bacteria were grown on Nutrient agar (Merck Millipore, Germany), micro-fungi were grown on Bengal rose agar (Merck Millipore, Germany), and actinomycetes were grown on Actinomycetes agar (HiMedia Laboratories, India). The number of colonies (colony forming units - CFUs) was calculated per 1 g of dry soil.

### **2.3.2. Total enzyme activity of the soil biota (FDA)**

The total activity of the microorganisms in the rhizosphere soil samples was hydrolyzed by fluorescein diacetate (FDA) according to SCHNÜRER and ROSSWALL (1982) and by ADAM and DUNCAN (2001) with minor modifications (VILLÁNYI et al. 2006).

### **2.3.3. Soil respiration studies**

#### **2.3.3.1. Basal- and substrate-induced respiration**

The activity of soil microorganisms was measured with basal (BRESP)- and substrate-induced respiration (SIR) techniques. From each sample 3 replicates were used (n=4 treatment).

Regarding BRESP, evolved CO<sub>2</sub> was measured after 4 and 24 h and the difference between them gave the rate of CO<sub>2</sub> production ( $\mu\text{g CO}_2\text{-C g soil}^{-1} \text{ h}^{-1}$ ). Substrate-induced respiration was also measured from the same soil sample by adding 200-200  $\mu\text{l}$  D-glucose solution (Reanal Labor, Budapest) (8 mg glucose g<sup>-1</sup> soil). The CO<sub>2</sub> formed during BRESP and SIR was measured with a gas chromatograph (GC 8000, Fisons, Rodano, Italy) (SZILI-KOVÁCS and TURKEY, 2005) and Clarity 4.0 software (DataApex Ltd., Prague, Czech Republic).

#### **2.3.3.2. Microrespiration measurement**

In the microrespiration experiment twenty-three different substrates and ultrapure distilled water (control) were distributed to each plate in four replications (subsamples per plate) (ANANYEVA et al. 2008). The pH of these substrate solutions were adjusted to 6.5 by 1 M NaOH or HCl solutions.

## **2.4. Molecular examination of soil microbial community**

### **2.4.1. Nucleic acid isolation from soil bacteria**

Microbial DNA was isolated from the spring soil samples with Soil Microbe DNA MiniPrep Kit (Zymo Research, Irvine, USA). The concentration of extracted DNA was measured using an ND 1000 NanoDrop™ spectrophotometer (Thermo Fisher Scientific, Wilmington, USA).

In addition to the determination of nucleic acid concentration (measured at  $\lambda=260$  nm) humic acids ( $\lambda=230$  nm) and protein ( $\lambda=280$  nm) were measured from the obtained absorbances (KÖDÖBÖCZ és MURÁNYI, 2012).

#### **2.4.1.1. Methodological development of bacterial DNA isolation from soil**

DNA was isolated from the autumn and spring soil samples with the above mentioned kit. Quantitative (concentration ng/ $\mu\text{l}$ ) and qualitative (humic acid 260/230 nm, protein 260/280 nm) soil DNA parameters were measured with ND 1000 NanoDrop™ spectrophotometer.

I aimed to determine whether there is a significant difference between the seasons in the examined parameters. I hypothesized that the efficiency of soil DNA extraction with Microbe DNA MiniPrep Kit (Zymo Research) could be improved by shaking. Therefore I tested four shaking methods. My aim was to improve the separation of soil aggregates and to improve the quantitative and qualitative indices of extracted DNA.

Based on the results of my preliminary experiment, using different physical soil management methods I have improved the quantity and quality of DNA with the most efficient



season (spring) and soil types (VO). I tested the following machines at maximum gear for three different periods of time:

A) homogenizing, FastPrep-24™, (mpbio™, Kuwait, Near East, 90-250 V, 50-60 Hz, 1200 W, 1-60 sec, speed 4,0-6,5 rev/sec, 1, 3, 5 min shaking time.

B) horizontal vortex, (Pulsing Vortex Mixers, VWR® International, Radnor, Pennsylvania, 120 V, 50-60 Hz, 3200 rev/min), 1, 5, 10 min shaking time.

C) cell mill, (Mini-Bead Beater-16 (MIDSCI), St. Louis, America, 115 V, 60 Hz, 3450 rev/min), 1, 3, 5 min shaking time.

D) vortex, (ZX3 Vortex Mixer, VELP Scientifica, Inc., Bohemia, United States, 100-240 V, 50-60 Hz, 3000 rev/min), 1, 5, 10 min shaking time.

The experiment was repeated with the most effective shaking times with each shaker for the other two soil types (AO, VHO). During the measurements I worked with 5 replicates. After shaking, I continued DNA extraction with the above kit with each sample following the manufacturer's instructions. After the quality and quantity parameters of the obtained DNA were measured with a ND-1000 NanoDrop™ spectrophotometer and fiber optic microcuvette (Hellma TrayCell®, Dialab Ltd., Budapest) spectrophotometer (Helios Beta, Thermo Spectronic, Thermo Fisher Scientific, Wilmington, USA).

#### **2.4.2. Soil Microbial DNA amplification by Nested - Polymerase Chain Reaction (PCR)**

For the analysis of the 16S rRNA gene section of the spring soil samples a PCR method was used. Partial 16S rRNA gene was amplified by a nested-PCR method in two steps. The first was carried out using R1494 and F203α Alphaproteobacteria specific primer pair (detection of nitrogen-fixing bacteria), followed by a second PCR with R1378 and F984GC universal bacterial primer pair (GOMES et al. 2001). The nested-PCR amplifications were performed in an ICYCLER Thermal Cycler machine (Bio-Rad Laboratories, Applied Biosystems, Foster, California, USA) in 25 µl.

Temperature profile of the first PCR included: initial denaturation (95°C, 4 min) followed by 30 cycles of denaturation (95°C, 30 s), annealing (56°C, 30 s) and extension (72°C, 1 min), with a single final extension (72°C, 10 min). The second PCR was carried out with almost the same temperature profile as the first one, except that the annealing temperature was at 53°C with F984GC; R1378 primer pair (Biocenter Laboratory Service Kft, Szeged). As a template, the PCR product amplified in the previous round was used. The size of the fragments obtained by electrophoresis separation of DNAs was determined using a DNA molecular weight marker (GeneRuler™ 1 kb Plus DNA Ladder, Thermo Scientific). Each PCR reaction was checked by electrophoresis in a 1.5% agarose gel (SeaKem LE Agarose (Lonza) containing 1xTAE (50x TAE Buffer (Tris-acetate-EDTA, Lonza, Belgium) buffer and nucleic acid staining solution (GR Safe Nucleic Acid Gel Stain, 10000x). PCR product was visualised under UV light.

#### **2.4.3. Amplification of the soil microbial *nifH* gene by polymerase chain reaction (PCR)**

The *nifH* gene of microbes in spring soil samples (n=4 per treatment) was detected by PCR. The primer pairs *nifH* FOR and *nifH* REV were used in the PCR reaction (12,5 µl) for the detection of nitrogen-fixing bacteria. The heat profile of the PCR reaction was the same as the

previously used universal bacterial primer. The PCR product separation of the *nifH* gene (~457 bp) was performed by agarose gel electrophoresis.

#### **2.4.4. Denaturing gradient gel electrophoresis (DGGE)**

For DGGE (Denaturate Gradient Gel Electrophoresis), the 16S rRNA gene fragment amplified by nested PCR was used. DGGE was performed in a 7% polyacrylamide (PAA) gel using INGENYphorU-2 electrophoresis system (Ingeny International BV, Netherlands) with HYBAID PS 250 running unit (Thermo Hybaid, USA).

TotalLab TL120 (TotalLab, England) software was used to evaluate the gel pattern. Dominant, well separated and intense bands were excised, and DNA was reextracted from the gel.

Using the 984GC forward and 1378 reverse primers amplification was done by PCR then the presence and quality of the PCR products was checked by 1.5% agarose gel electrophoresis.

#### **2.4.5. Sequencing by capillary electrophoresis**

We used the Sanger sequencing laboratory of the Hungarian Academy of Sciences, Szeged Biological Research Center to determine the sequence of the PCR products. Sanger sequencing of the DNA was performed with 3500 Series Genetic Analyzer (Life Technologies) in the Biological Research Centre, Szeged (Hungary).

### **2.5. Statistical analysis**

The data obtained in the experiments were evaluated by Microsoft Office Excel 2016 software.

Outliers were selected. SPSS 25.0. program package (IBM Corp., Armonk, NY, USA) was used for the statistical analysis. 95% confidence level ( $p \leq 0,05$ ) was chosen to the data evaluation. Data normality and homogeneity of deviation was examined. Levenne-test was applied to examine the homogeneity of deviation while Shapiro-Wilk-test was used to examine the normality. Single-factor analysis of variance (ANOVA) was used when comparing the results. At the pairwise comparisons of treatments Tukey HSD post hoc test was applied in case of homogeneity of deviation while Games-Howell test was used in case of inhomogeneity of deviation.

For the methodological development of soil bacterial nucleic acid isolation, SRD software was used for multicriterial statistical evaluation of several performance parameters taking into account several parameters simultaneously.

The characterization of the relationship between the physical, chemical and microbiological properties of the main soils was calculated by Pearson's correlation coefficient.

To evaluate to the multicriterial performance indicators, statistical evaluations were performed by SRD software and Statistica 12.0 program package. Multicriteria comparison for the two seasons was made by RV coefficient

### 3. RESULTS

#### 3.1. Results of status tests

##### 3.1.1. Electrical conductivity

Significant difference was detected in the EC value between the seasons (season:  $F(1;125)=63,3$ ;  $p\leq 0,001$ ), soil type/management methods (soil:  $F(1;125)=112$ ;  $p\leq 0,001$ ) and the soil type/management methods and between season interaction (soil\*season  $F(1;125)=22,8$ ;  $p\leq 0,001$ ). In autumn 2011, the highest EC values were in AVK soil ( $0,418\pm 0,128$  mS/cm), the lowest in HK ( $0,057\pm 0,014$  mS/cm). In spring 2012, the highest EC value was provided by VK ( $0,258\pm 0,038$  mS/cm), while the smallest was in the case of the autumn HK ( $0,027\pm 0,006$  mS/cm).

##### 3.1.2. $pH_{H_2O}$ -values

In contrast of the soil types/management methods (soil:  $F(1;129)=282$ ;  $p\leq 0,001$ ) significant difference was not detected in case of total  $pH_{H_2O}$ -values between seasons (season:  $F(1;129)=1,16$ ;  $p=0,284$ ) and soil type/management methods and between season interaction (soil\*season:  $F(1;129)=1,57$ ;  $p=0,170$ ). In autumn, 2011 VK soil type ( $7,85\pm 0,12$ ) had the the highest  $pH_{H_2O}$  and HK ( $5,45\pm 0,24$ ) the lowest. In spring, 2012 the highest  $pH_{H_2O}$  value was found in the VO soil type ( $7,93\pm 0,14$ ), and the lowest one in HK ( $5,40\pm 0,11$ ) similarly to the autumn.

##### 3.1.3. Nitrogen forms, humus and carbon content

$NH_4^+$ -N values showed significant difference between seasons (season:  $F(1;123)=31,7$ ;  $p\leq 0,001$ ), soil type/management methods (soil:  $F(1;123)=22,6$ ;  $p\leq 0,001$ ), soil type/management methods and between season interaction (soil\*season  $F(1;123)=10,3$ ;  $p\leq 0,001$ ). In autumn 2011, the highest  $NH_4^+$ -N value was found in AVK soil ( $5,33\pm 2,23$  mg/kg), while the lowest was in HK ( $2,06\pm 1,25$  mg/kg). In spring, the highest peak was observed for AVK, which was extremely high ( $15,9\pm 8,9$  mg/kg) the lowest value was at VHO ( $2,54\pm 0,91$  mg/kg).

The  $NO_3^-$ -N level also showed a significant difference between the seasons (season:  $F(1;129)=70,9$ ;  $p\leq 0,001$ ), soil type/management methods (soil:  $F(1;129)=17,9$ ;  $p\leq 0,001$ ), soil type/management methods and between season interaction (soil\*season:  $F(1;129)=22,0$ ;  $p\leq 0,001$ ). In autumn, the highest value was found in the VO soil type ( $34,4\pm 19,2$  mg/kg), the lowest value was for the AO soil type ( $7,05\pm 1,87$  mg/kg). In spring, the highest was observed in VK ( $40,2\pm 17,9$  mg/kg), the lowest in HK ( $1,19\pm 0,44$  mg/kg).

Considering the total nitrogen significant difference was observed between the two seasons (season:  $F(1;131)=16,5$ ;  $p\leq 0,001$ ) and soil type/management methods (soil:  $F(1;131)=961$ ;  $p\leq 0,001$ ). In contrast, there was no difference in the interaction between soil types/management methods and between season interaction (soil\*season:  $F(1;131)=2,20$ ;  $p=0,058$ ). The highest total nitrogen values in autumn 2011 were measured at AO soil ( $0,219\pm 0,009\%$ ) and the lowest at HK ( $0,059\pm 0,005\%$ ). Spring, 2012 values also showed a similar trend, with the highest values also at AO ( $0,207\pm 0,006\%$ ) and the lowest also at HK ( $0,045\pm 0,016\%$ ).

Humus content didn't show significant difference between seasons (season:  $F(1;132)=0,615$ ;  $p=0,434$ ), however significant difference was found in soil type/management methods (soil:  $F(1;132)=915$ ;  $p\leq 0,001$ ) and soil types/management methods and between season interaction (soil\*season:  $F(1;132)=2,32$ ;  $p\leq 0,001$ ). The AO soil provided the highest autumn humus value ( $4,07\pm 0,30\%$ ) and the VHO the lowest ( $0,905\pm 0,240\%$ ). The spring trend was almost the same, AO ( $4,04\pm 0,17\%$ ) and HK ( $0,717\pm 0,130\%$ ) were the two extremes.

No significant differences were found in the carbon content between the seasons (season:  $F(1;131)=0,247$ ;  $p=0,620$ ), only in the case of different soil type/management methods (soil:  $F(1;131)=1003$ ;  $p\leq 0,001$ ) and soil types/management methods and between season interaction (soil\*season  $F(1;131)=2,79$ ;  $p\leq 0,001$ ). In autumn, the highest carbon content was produced by AO ( $2,32\pm 0,11\%$ ) and the lowest by VHO ( $0,525\pm 0,137\%$ ). Compared to this, a similar tendency was observed in the case of AO ( $2,34\pm 0,10\%$ ) and HK ( $0,415\pm 0,076\%$ ). Total nitrogen, humus and carbon content tend to decrease from clayey soil to sandy.

#### **3.1.4. AL-soluble nutrient contents (Ca, Na, P<sub>2</sub>O<sub>5</sub>, K<sub>2</sub>O)**

According to AL-Ca values, there was no significant difference between the seasons (season:  $F(1;101)=0,015$ ;  $p=0,903$ ), soil types/management methods or between season interaction (soil\*season  $F(1;101)=0,094$ ;  $p=0,993$ ), however in case of soil types/management methods there was significant difference (soil:  $F(1;101)=122$ ;  $p\leq 0,001$ ). In autumn, the highest values were observed for VO ( $16790\pm 5521$  mg/kg) and the lowest for HK ( $317\pm 49$ ), similar to the spring values: VO ( $16295\pm 5219$  mg/kg), HK ( $317\pm 49$  mg/kg).

AL-Na values were indicated significant differences between the different seasons (season:  $F(1;115)=39,8$ ;  $p\leq 0,001$ ), soil types/management methods (soil:  $F(1;115)=193$ ;  $p\leq 0,001$ ), soil types/management methods and between season interaction (soil\*season:  $F(1;115)=2,44$ ;  $p\leq 0,001$ ). Among the autumn 2011 samples, the highest AL-Na value was measured at AO ( $52,5\pm 14,6$  mg/kg), the lowest was provided by HK ( $8,16\pm 1,83$  mg/kg). In spring, the same trend was observed for AO ( $53,2\pm 13,0$  mg/kg) and HK ( $2,28\pm 1,03$  mg/kg) averages. In both seasons significantly higher values of the AO was measured compared to the average of the other values.

In case of AL-P<sub>2</sub>O<sub>5</sub> values a significant difference was observed between soil types/management methods (soil:  $F(5;112)=543$ ;  $p\leq 0,001$ ) but no such difference was found between the seasons (season:  $F(1;112)=1,17$ ;  $p=0,280$ ), soil types/management methods or between season interaction (soil\*season:  $F(5;112)=1,96$ ;  $p=0,089$ ). The highest values of both seasons were given by VO soil, in autumn ( $594\pm 49$  mg/kg) and in spring ( $610\pm 51$  mg/kg). The lowest values were consistently assigned to AO soils (autumn:  $75,9\pm 18,9$  mg/kg), (spring:  $68,6\pm 15,7$  mg/kg).

AL-K<sub>2</sub>O data shown significant difference between and soil types/management methods (soil:  $F(5;122)=211$ ;  $p\leq 0,001$ ), soil types/management methods and between season interaction (soil\*season:  $F(5;122)=16,7$ ;  $p\leq 0,001$ ). No significant difference was detected between the seasons (season:  $F(1;122)=3,93$ ;  $p=0,051$ ). According to the autumn tendency, the highest value was found in VO soil ( $540\pm 74$  mg/kg), while the lowest value was detected in VHO soil ( $167\pm 62$  mg/kg). Autumn values were almost the same as spring (spring: VK  $655\pm 64$  mg/kg; VHO:  $163\pm 29$  mg/kg).

## 3.2. Results of functional tests

### 3.2.1. Investigation of soil microbiology (CFU)

According to the *Actinomycetes* CFU significant difference was found between seasons (season:  $F(1;128)=252$ ;  $p\leq 0,001$ ), soil types/management methods (soil:  $F(1;128)=91,7$ ;  $p\leq 0,001$ ), soil types/management methods and between season interaction (soil\*season:  $F(1;128)=15,2$ ;  $p\leq 0,001$ ). In autumn, the highest CFU values were observed in the AVK soil sample ( $7,91\pm 0,23$  logCFU/g dry soil), while the lowest was observed in the HK ( $6,39\pm 0,20$  logCFU/g dry soil). In the spring, however, the maximum value was presented by VO ( $8,39\pm 0,16$  logCFU/g dry soil) and the minimum by VHO ( $7,33\pm 0,26$  logCFU/g dry soil).

As for *Bacillus* sp. CFU significant differences were found between seasons (season:  $F(1; 99)=15,2$ ;  $p\leq 0,001$ ) and soil types/management methods (soil:  $F(1; 99)=48,6$ ;  $p\leq 0,001$ ), and interaction between soil types/land management methods (soil\*season:  $F(1; 99)=19,7$ ;  $p\leq 0,001$ ). In case of HK soil,  $10^4$  germs were detected in autumn and spring, per 1 g soil. In the case of spring, the measured  $n=2$  data is not suitable for statistical analysis, therefore the two years are not statistically comparable. The probable cause of low CFU is acidic, nutrient-poor sandy soil. Regarding the autumn data ( $n=7$ ) it was found that there was no significant difference between the soils\*management method, but there was a significant difference between the different soils ( $p\leq 0,001$ ). In the autumn the highest value was provided by VO soil ( $4,41\pm 0,18$  logCFU/g dry soil) and the lowest value was by AO ( $4,08\pm 0,18$  logCFU/g dry soil), while in the spring, the maximum value was for VHO ( $4,80\pm 0,40$  logCFU/g dry soil) and the minimum was for AO ( $3,41\pm 0,32$  logCFU/g dry soil).

Filiform fungi CFU showed significant difference between the seasons (season:  $F(1;132)=143$ ;  $p\leq 0,001$ ) and soil types/management methods (soil:  $F(1;132)=106$ ;  $p\leq 0,001$ ), soil types/management methods and between season interaction (soil\*season:  $F(1;132)=24,0$ ;  $p\leq 0,001$ ). In the autumn, the highest germination values were reported by AO and AVK ( $5,64\pm 0,15/0,36$  logCFU/g dry soil), compared to the lowest by VHO ( $4,27\pm 0,24$  logCFU/g dry soil). In spring I measured the maximum value for VO soil ( $5,70\pm 0,13$  logCFU/g dry soil), and the minimum was for the VHO soil ( $4,85\pm 0,27$  logCFU/g dry soil).

Similar to the above tendency spore forming CFU showed significant difference between the seasons (season:  $F(1;120)=10,0$ ;  $p\leq 0,001$ ) and soil types/management methods (soil:  $F(1;120)=141$ ;  $p\leq 0,001$ ) and soil types/management methods and between season interaction (soil\*season:  $F(1;120)=78,9$ ;  $p\leq 0,001$ ). In autumn 2011, the most significant germination values were provided by AO ( $6,51\pm 0,12$  logCFU/g dry soil), while the lowest ones were provided by VHO ( $4,30\pm 0,27$  logCFU/g dry soil). In comparison, in the spring season the trend was the following: max: VO  $5,52\pm 0,10$  logCFU/g dry soil, min.: HK  $4,45\pm 0,30$  logCFU/g dry soil. The results of the spring sampling tend to be the same as those of the autumn sampling, but there is a significant difference in their value in each case.

### 3.2.2. Soil enzyme activity (FDA)

Based on the FDA results significant difference was not found between the seasons (season:  $F(1;127)=0,013$ ;  $p=0,908$ ) while significant differences was detected at soil types/management methods (soil:  $F(1;127)=77,2$ ;  $p\leq 0,001$ ) and soil types/management methods and between season interaction (soil\*season:  $F(1;127)=13,3$ ;  $p\leq 0,001$ ). In the two seasons the same soil type and farming method (AO) gave the highest (max.: autumn:  $96,3\pm 28,5$   $\mu\text{gFl/g soil/h}$ ;

spring:  $59,2 \pm 23,4 \mu\text{gFl/g soil/h}$ ) and the lowest (VHO) values (min: autumn:  $11,1 \pm 5,4 \mu\text{gFl/g soil/hour}$ ; spring:  $13,8 \pm 6,5 \mu\text{gFl/g soil/hour}$ ).

### **3.2.3. Soil respiration**

#### **3.2.3.1. Basal and substrate induced respiration**

During the experiments of BRESP significant difference was detected between seasons (season:  $F(1;34)=135$ ;  $p \leq 0,001$ ) soil types/management methods (soil:  $F(1;34)=4,05$ ;  $p \leq 0,001$ ), and soil types/management methods and between season interaction (soil\*season:  $F(1;34)=3,41$ ;  $p \leq 0,001$ ). In autumn, the highest BRESP values were given by AVK ( $0,330 \pm 0,163 \mu\text{g CO}_2\text{-C/g soil/h}$ ) and the lowest by VK soil ( $0,044 \pm 0,007 \mu\text{g CO}_2\text{-C/g soil/h}$ ). In spring the values were significantly higher (max: VO  $0,814 \pm 0,202 \mu\text{g CO}_2\text{-C/g soil/h}$ ; min: VK  $0,448 \pm 0,133 \mu\text{g CO}_2\text{-C/g soil/h}$ ).

Similar to BRESP, SIR values showed the same significant tendency: (season:  $F(1;36)=134$ ;  $p \leq 0,001$ ), (soil:  $F(1;36)=147$ ;  $p \leq 0,001$ ), (soil\*season:  $F(1;36)=21,3$ ;  $p \leq 0,001$ ). The highest SIR in autumn was in AO soil ( $6,46 \pm 0,39 \mu\text{g CO}_2\text{-C/g soil/h}$ ), while the lowest was observed in VHO ( $0,45 \pm 0,14 \mu\text{g CO}_2\text{-C/g soil/hour}$ ). In spring, the highest SIR activity was observed in VO soil ( $7,74 \pm 1,05 \mu\text{g CO}_2\text{-C/g soil/h}$ ), and the lowest value was in VHO soil ( $1,81 \pm 0,40 \mu\text{g CO}_2\text{-C/g soil/hour}$ ).

#### **3.2.3.2. Microrespiration**

Out of the 23 total substrates tested, citrate provided the highest values in both seasons, while lysine provided the lowest values.

As for citrate significant difference was detected at soil types/management methods (soil:  $F(1;36)=4,51$ ;  $p \leq 0,001$ ), but was no significant difference between the seasons (season:  $F(1;36)=0,957$ ;  $p=0,334$ ) and soil types/management methods and between season interaction (soil\*season:  $F(1;36)=1,11$ ;  $p=0,369$ ). In autumn, the highest citrate activity response to citrate was found in AVK soil ( $1,84 \pm 0,21 \mu\text{g CO}_2\text{-C/g soil/h}$ ), while the lowest was provided by VO ( $0,985 \pm 0,550 \mu\text{g CO}_2\text{-C/g soil/h}$ ). In spring higher activity was detected, which was not significant. The highest value was measured at AO ( $2,01 \pm 0,31 \mu\text{g CO}_2\text{-C/g soil/h}$ ), while the lowest value was found in VO ( $1,13 \pm 0,32 \mu\text{g CO}_2\text{-C/g soil/hour}$ ).

During the experiment of lysine significant difference was found between the seasons (season:  $F(1;36)=50,5$ ;  $p \leq 0,001$ ) at soil types/management methods (soil:  $F(1;36)=11,5$ ;  $p \leq 0,001$ ) soil types/management methods and between season interaction (soil\*season:  $F(1;36)=3,37$ ;  $p \leq 0,001$ ). During the autumn, HK soil showed the highest catabolic activity ( $0,708 \pm 0,035 \mu\text{g CO}_2\text{-C/g soil/h}$ ), whereas the lowest value was provided by AO ( $0,345 \pm 0,137 \mu\text{g CO}_2\text{-C/g soil/h}$ ). In comparison, VHO had the highest activity in spring ( $0,501 \pm 0,033 \mu\text{g CO}_2\text{-C/g soil/h}$ ) and lowest AO, similarly to autumn ( $0,309 \pm 0,032 \mu\text{g CO}_2\text{-C/g soil/h}$ ).

### **3.3. Multicriteria complex evaluation of major soil physico-chemical and microbiological parameters**

The Pearson's correlation coefficient was used to characterize the relationship between the main soil physics, chemistry and microbiology parameters. In autumn, the strongest significant correlation ( $r=0,937$ ) (ref.: very strong correlation range  $r=0,80-1,00$ ) for organic carbon (%) and

SIR ( $\mu\text{g CO}_2\text{-C/g soil/h}$ ), and humus content (%) and SIR ( $\mu\text{g CO}_2\text{-C/g soil/h}$ ). This tendency is due to the fact that the humus content is calculated from the organic carbon value, as shown by the correlation between them ( $r=0,999$ ). While the weakest correlation ( $r=-0,514$ ) (ref.: medium correlation range  $r=0,40-0,59$ ) was found between  $\text{pH}_{\text{H}_2\text{O}}$  and the catabolic activity of citrate substrate ( $\mu\text{g CO}_2\text{-C/g soil/h}$ ). In spring, the strongest correlation was found between  $\text{pH}_{\text{H}_2\text{O}}$  and AL-Na (mg/kg) ( $r=0,871$ ) (ref.: very strong correlation range  $r=0,80-1,00$ ). In contrast, the lowest significant correlation was found between BRESP ( $\mu\text{g CO}_2\text{-C/g soil/h}$ ) and citrate substrate catabolic activity ( $\mu\text{g CO}_2\text{-C/g soil/h}$ ) (ref.=medium), (correlation range  $r=0,40-0,59$ ).

For the above parameters I made a multicriteria comparison using the RV coefficient for the autumn (matrix A) and the spring (matrix B) seasons. Result was that the correlation similarity between the two seasons (matrix) variables is significant ( $p<0,001$ ).

### **3.4. Investigation of soil microbial community diversity**

#### **3.4.1. Quantitative and qualitative parameters of soil bacterial nucleic acid isolation**

Concerning DNA concentration, significant difference was detected in the different seasons (season:  $F(1;130)=9,84$ ;  $p\leq 0,001$ ) at soil types/management methods (soil:  $F(1;130)=3,44$ ;  $p\leq 0,001$ ), but significant difference was not detected at soil types/management methods and between season interaction (soil\*season:  $F(1;130)=2,24$ ;  $p=0,054$ ). The highest nucleic acid concentration was associated with HK soil in the autumn ( $28,4\pm 8,4$  ng/ $\mu\text{l}$ ), while the lowest value was provided by VK ( $20,7\pm 3,6$  ng/ $\mu\text{l}$ ). In spring, however, the best result was observed at VO ( $34,3\pm 11,1$  ng/ $\mu\text{l}$ ) and the lowest was at AO ( $24,5\pm 3,8$  ng/ $\mu\text{l}$ ).

In terms of protein content significant difference was detected at soil types/management methods (soil:  $F(1;132)=3,58$ ;  $p\leq 0,001$ ), in contrast difference was not detected at soil types/management methods and between season interaction (soil\*season:  $F(1;132)=1,15$ ;  $p=0,336$ ). In autumn, the purest protein-containing sample was found at VO ( $1,23\pm 0,09$  260/280 nm, ref.:  $> 1,8$ ; 260/280 nm), while the most contaminated sample was detected at AO ( $1,08\pm 0,10$  260/280 nm). Similarly to autumn, VO gave the purest value ( $1,11\pm 0,14$  260/280 nm) in the spring period, while VK ( $0,992\pm 0,101$  260/280 nm) provided the most polluted value.

Humic acid indicated that significant difference was not detected between different seasons (season:  $F(1;132)=0,646$ ;  $p=0,423$ ) however significant difference was detected at soil types / management methods (soil:  $F(1;132)=10,3$ ;  $p\leq 0,001$ ) and soil types/management methods and between season interaction (soil\*season:  $F(1;132)=2,73$ ;  $p\leq 0,001$ ). The highest detected purity and most contaminated soils during the two seasons were the same (autumn: max.: VO  $0,793\pm 0,330$  260/230 nm (ref.:  $> 2$  260/230 nm)); min.: VK  $0,493\pm 0,088$  260/230 nm; spring: max.: VO  $0,663\pm 0,096$  260/230 nm, min.: VK  $0,549\pm 0,057$  260/230 nm). Overall spring VO soil had the highest DNA concentration with the best protein and humic acid levels measured in both seasons. At the lowest concentration of nucleic acid (VK  $20,7\pm 3,6$  ng/ $\mu\text{l}$  in autumn) the most polluted protein (autumn AO  $1,08$  260/280 nm) and humic acid values (autumn VK  $0,493\pm 0,088$  260/230 nm) connected.

##### **3.4.1.1. Multicriteria complex examination of quantitative and qualitative parameters of soil DNA isolation**

Based on the DNA parameters of the two examined seasons I created the theoretically best performing soil type and cultivation method using the SRD statistical method. The obtained DNA isolation method can be classified into groups of nucleic acid indicators according to their

efficiency rankings. VO and AO gave the best DNA yields, VK, AVK and VHO were the second most efficient group, while HK was the least efficient.

### **3.4.1.2. Methodological development of bacterial DNA isolation from soil**

Based on the SRD analysis the VO soil type performed best in terms of the quantity and quality parameters of the nucleic acid. Based on this soil sample a methodological development was made improving the quantity and quality indicators during DNA isolation. Quantitative and qualitative indices of DNA show a heterogeneous pattern on the VO soil type, depending on which is the most effective and least effective according to the 3 shaking times, the 4 shaking devices and the 2 spectrophotometers.

The most successful shaking was provided by the cell mill instrument with a shaking time of 3 minutes, with both nucleic acid concentration ( $51,5 \pm 23,0$  ng/ $\mu$ l as measured by TrayCell<sup>®</sup> microcuvette) and quality parameters (humic acid value detected by NanoDrop<sup>™</sup>  $0,638 \pm 0,021$  260/230 nm (NanoDrop<sup>™</sup> protein content  $1,20 \pm 0,04$  260/280 nm) also proved to be the best.

In contrast, the lowest values were obtained by the Pulsing Vortex shaker at 5 minutes (measured with TrayCell<sup>®</sup> microcuvette: DNA concentration  $10,8 \pm 3,5$  ng/ $\mu$ l; humic acid content  $0,140 \pm 0,035$  at 260/230 nm; detected protein value  $0,586 \pm 0,080$  260/280 nm).

I extended the experiment to 3 soil types (AO, VHO, VO). The 4 types of shakers were the same (BB, FP, PV, ZX) where I tested the parameters with the TrayCell<sup>®</sup> microcuvette and NanoDrop<sup>™</sup> spectrophotometer to determine which soil type was most effective. The shaking time was considered to be the most efficient minutes/shaker ever measured.

The highest total DNA concentration was obtained by vortexing for 10 minutes on a VHO soil ( $64,5 \pm 14,2$  ng/ $\mu$ l) as measured by a TrayCell<sup>®</sup> spectrophotometer, while the lowest concentration on AO soil ( $7,58 \pm 2,03$  ng/ $\mu$ l) was observed by pulsing Vortex for 1 minute measured with a NanoDrop<sup>™</sup>.

The total humic acid content was as follows, with the FastPrep shaker, after 5 minutes homogenization with NanoDrop<sup>™</sup>, yielding the clearest value for VHO soil ( $1,77 \pm 0,34$  260/230 nm), the lowest compared to Pulsing Vortex on VO soil at 1 minute (as measured by TrayCell<sup>®</sup> microcuvette)  $0,164 \pm 0,011$  260/230 nm.

The purest protein content was, according to the cumulative evaluation, obtained with 5 min AO soil as measured with a NanoDrop<sup>™</sup> ( $1,64 \pm 0,52$  260/280 nm) and lowest with Pulsing Vortex as measured with a TrayCell<sup>®</sup> microcuvette for 1 minute ( $0,652 \pm 0,127$ ) 260/280 nm.

There was a significant difference between the isolated nucleic acid parameters of each soil type using each spectrophotometer. An exception to this was the protein content measured with NanoDrop<sup>™</sup>, where no significant difference was found.

### **3.4.1.3. Extended multicriteria complex examination of quantitative and qualitative parameters of DNA isolation from soil**

Correlation analysis was performed on the methodological development data of soil bacterial nucleic acid isolation. Based on the different shaking devices tested, the shaking times, the soil types' performances and the DNA parameters, I created the theoretically best performing method using the SRD statistical method. Best shaking methods: 10 minutes vortexing with AO and VHO soils. The second most effective group was 10 minutes vortex shaking on VO soil, 3 minutes cell mill AO soil and 1 minute Pulsing Vortex AO and VHO soils, and the least effective was 5 minutes vortex shaking on VO soil.

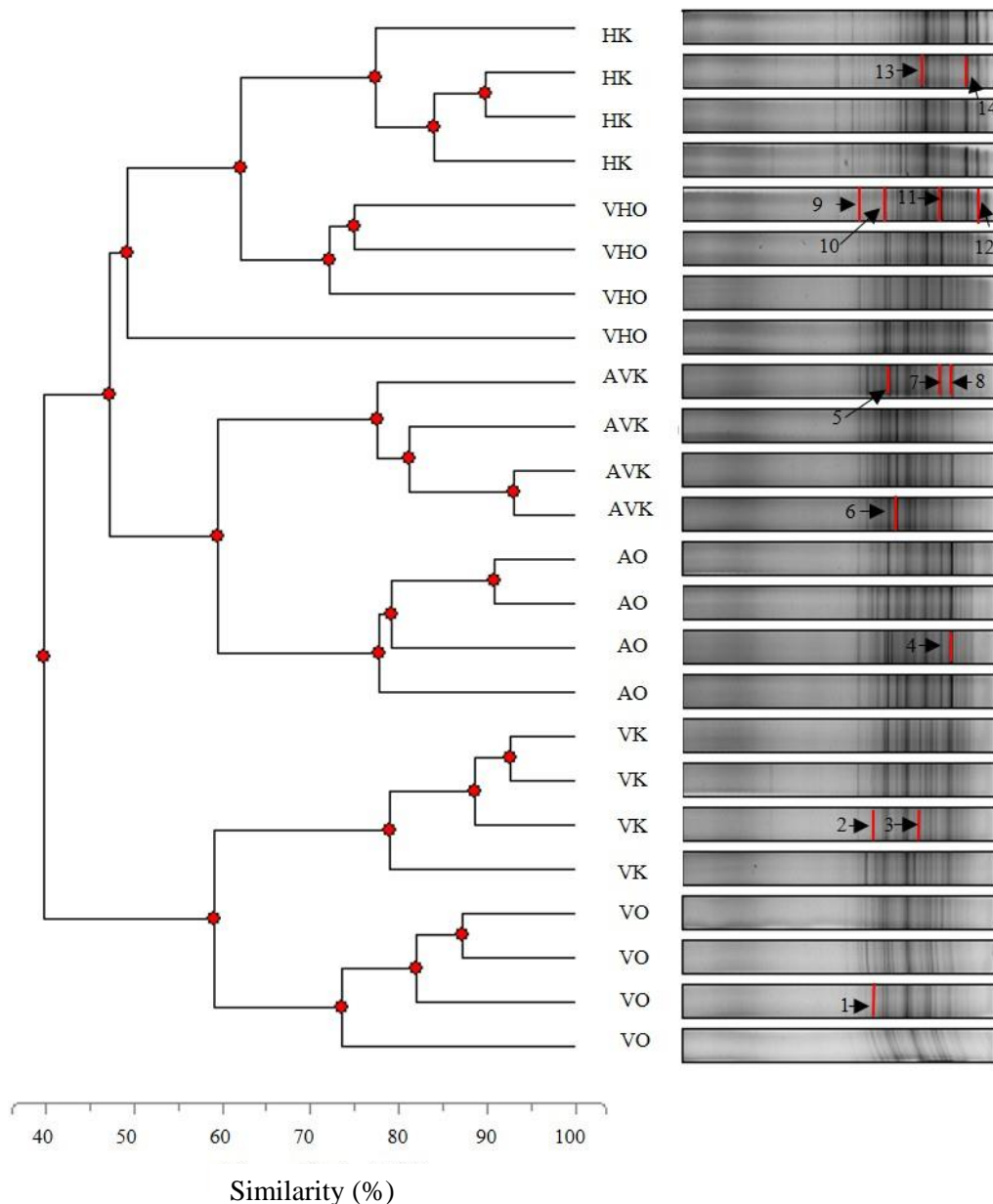


#### 3.4.1.4. Detection of soil microbial community by PCR and nested PCR

Soil bacteria were detected by nested-PCR (universal and  $\alpha$ -proteobacteria). I got the expected 500 bp PCR products for the spring samples. For the *nifH* genes, I obtained the desired 457 bp product for the above samples.

#### 3.4.2. Denaturing gradient gel electrophoresis (DGGE)

According to the DGGE patterns  $\alpha$ -proteobacteria community structures of the three soil types formed separate groups (**Figure 1.**). The first distinct group (at 40% similarity) comprised the community structures of loamy soil samples. The second branching (at 47% similarity) separated the community fingerprints of the clay and the sandy soils. The smallest spatial heterogeneity was typical for loamy soil and the largest was revealed in the sandy soil samples (VHO). Separation of the community patterns of conventional and organic soil samples was observed at 60–65% similarity in almost every case. The bacterial diversity indices did not differ significantly in the DGGE band pattern ( $H' p=0,252$ ,  $E' p=0,498$ ). However, conventional soils had the highest bacterial diversity compared to organic. The most diverse soil type was the HK soil ( $H'=2,82$ ;  $E=0,89$ ;  $R=23$ ). The lowest diversity was given by AO ( $H'=2,46$ ;  $E=0,87$ ;  $R=17$ ), but the AVK showed a higher value ( $H'=2,65$ ;  $E=0,87$ ;  $R=20$ ). The loam soil had medium diversity indices for VK ( $H'=2,68$ ;  $E=0,89$ ;  $R=19$ ) and VO ( $H'=2,59$ ;  $E=0,87$ ;  $R=19$ ).



*Legend: O=organic management method, K=conventional management method, A=clay soil (Karcag), AV=clay loam soil (Karcag), V=loamy soil (Martonvásár), VH=loamy sandy soil (Nyíregyháza), H=sandy soil (Nyíregyháza).*

**Figure 1.** The banding pattern of the DGGE UPGMA dendrogram of bacterial communities.

### 3.4.3. Sequencing

Fourteen distinct strong bands from the DGGE band pattern were sequenced and evaluated. It was confirmed that, based on sequence analysis of the 16S rRNA gene, all of the closest related bacteria identified as members of the  $\alpha$ -proteobacterial class. Sequencing the DNA isolated from soil samples,  $\alpha$ -proteobacteria isolates were identified to species rank 98,7 % (CHUN et al. 2018) using NCBI database - (*Pseudovibrio denitrificans* (VO); *Devosia* sp., *Ancylobacter rudongensis* (VK); *Devosia insulae* (AVK); *Rhizobium* sp. (HK). These species were detected which are the key indicators of the nitrogen cycle. They play an important role in organic decomposition and nitrogen mineralization. Nitrogen fixing bacteria was successfully detected by the presence of *nifH* gene.

**Table 1.:** The closest phylogenetic relatives of 16S rRNA gene sequences isolated from DGGE banding patterns.

<i>Sample</i>	<i>Band</i>	<i>Closest phylogenetic relatives<sup>I</sup></i>	<i>Similarity (%)</i>	<i>Habitat</i>	<i>Accession Nr.</i>
VO	1	<i>Pseudovibrio denitrificans</i>	99	Marine sponge	JF281743
VK	2	<i>Devosia</i> sp.	99	Natural Euprates poplar forests	KC464823
	3	<i>Ancylobacter rudongensis</i>	99,51	Roots of <i>Spartina anglica</i>	AY056830
AO	4	<i>Azospirillum</i> sp.	95,78	Soil	KT619165.1
AVK	5	<i>Devosia lucknowensis</i>	96	Soil	NR132697.1
	6	<i>Devosia insulae</i>	99,52	Soil	EF012357
	8	<i>Azospirillum</i> sp.	97,41	Soil	JF340293.1
VHO	9	<i>Mesorhizobium</i> sp.	96	Root bulb <sup>I</sup>	KJ000995
	10	<i>Rhizobium leguminosarum</i>	96	Soil	GU201843.1
	11	<i>Rhizobiales</i>	96,07	Soil	AB257851.1
HK	13	<i>Rhizobium</i> sp.	99	Root bulb <sup>II</sup>	KX097067
AVK	7	<i>Devosia</i> sp.	98	Soil	FN600566.2
VHO	12	<i>Rhodospirillales</i>	98	Soil	MG722008.1
HK	14	<i>Rhizobium</i> sp.	99	Root bulb <sup>II</sup>	KX097067.1

Legend: O=organic management method, K=conventional management method, A=clay soil (Karcag), AV=clay loam soil (Karcag), V=loamy soil (Martonvásár), VH=loamy sandy soil (Nyíregyháza), H=sandy soil (Nyíregyháza). Similarity (%)=same number of bases / length of complete overlap sequence. <sup>I</sup>=*Rhynchosia aurea*, <sup>II</sup>=*Trifolium* sp.

#### 4. NEW SCIENTIFIC RESULTS

1. I examined the composition and function of the soil microbial community in the organic and conventional management methods of three Hungarian soil types (Karcag - clay, clayey loam; Martonvásár - loamy soil; Nyíregyháza - sandy soils). Soil microbial community were examined by classical status and functional studies, taking into account the seasonal effects. I created a multicriteria reference database.

2. I found significantly higher values ( $p \leq 0,05$ ) in organic management methods than in conventional at the main soil chemical and microbiological parameters: total N (%), humus content (%), AL-Ca content (mg/kg), FDA ( $\mu\text{gFl/g soil/hour}$ ), CFU – *Bacillus* sp., filiform fungi, spore forming bacterium ( $\log\text{CFU/g dry soil}$ ), SIR-content ( $\mu\text{g CO}_2\text{-C/g soil/hour}$ ), MicroResp<sup>TM</sup> ( $\mu\text{g CO}_2\text{-C/g soil/hour}$ ) and the major and meso elements (Mg, B, Cu, Zn (mg/kg)). I proved that organic management method had a positive effect on soil quality and fertility, thus indirectly on soil microbial activity and community.

(GAZDAG et al. *Microbes and Environments*, 34 (3) 234-243. p., IF=2,47, Q1)

3. Methodological development was carried out to improve the quantitative and qualitative parameters of the extracted soil DNA during soil-specific DNA isolation. I analyzed the parameters of the DNA efficiency originated from the tested soils with complex assessment that takes several aspects into account (sum of SRD rankings). Considering soil type, shaker type and the shaking time the 10-minute vortex shaking was the most effective for organic clay and sandy loam soil.

4. There were significant differences in the sensitivity of the indicators used to characterize the above soils. Functional studies of soil microbial communities in soils can detect changes in management patterns – with particular regard to the MicroRespiration (MicroResp<sup>TM</sup>) method – between organic and conventional management soils based on pattern analysis of the degradative activity of microbial communities.

(GAZDAG et al. *Microbes and Environments*, 34 (3) 234-243. p., IF=2,47, Q1)

5. The molecular fingerprinting method (DGGE) of the investigated  $\alpha$ -proteobacterial communities in the investigated soils confirmed that the organic constituents of organic and conventional soils are different (60-65% similarity level). Based on sequence analysis of the 16S rRNA gene sequence, each of the closest related bacteria identified belonged to the  $\alpha$ -proteobacterial class. Six of the detected bacteria were identified at species level by CHUN et al. (2018) based on the 98,7% similarity threshold for 16S rDNA (*Pseudovibrio denitrificans* (VO); *Devosia* sp., *Ancylobacter rudongensis* (VK); *Deviosa insulae* (AVK); *Rhizobium* sp. (HK)). I found that the different soil types and vegetation had a more significant effect on the composition/structure of  $\alpha$ -proteobacterial communities than the different land management methods.

(GAZDAG et al. *Acta Agriculturae Scandinavica*, 69 (2), 147-154. p., IF=0,81, Q2)

## 5. CONCLUSIONS AND PROPOSALS

Comparing the main soil chemical parameters of different soil types and farming methods with the soil supply categories, the soils are classified according to salinity into the non-saline category (max.:  $0,418 \pm 0,128$  mS/cm, min.:  $0,027 \pm 0,006$  mS/cm) ( $<2$  mS/cm;  $<0,1\%$ ). This does not inhibit the development of crop plants, the irrigation water does not cause harmful salt accumulation in the examined soils (KÁTAI 2011). The  $\text{pH}_{\text{H}_2\text{O}}$  ranged from acidic (HK), weakly acidic (VHO) and neutral (AVK) to the weakly alkaline range (AO, VK) (KÁTAI, 2011) within the following interval (max.: spring VO) soil  $7,93 \pm 0,14$ ; min.: spring HK  $5,40 \pm 0,11$ ).

Most of the tested pH values were significantly higher in favor of organic soils, but the values were still within the optimal limits for soil bacteria (JEFFERY et al. 2010). The pH of each treatment was unchanged over the two seasons. Significant difference was not found between different seasons and soil types/management methods and between season interaction, but significant difference was found at seasons and soil types/management methods. Lower pH values in conventional areas are due to the acidifying effect of fertilizers (GAZDAG et al. 2019a).

The nitrogen results obtained are due to well-formed crop rotations because Leguminous plants were grown in all experimental areas except AO and VK. Nitrogen forms derived from them were slower to digest, thus providing a continuous supply of nitrogen to the cultivated plants, which satisfied the nitrogen needs of the crop's. The reason for the lower humus values in conventional areas is probably due to the limited organic matter content, as these plots did not have green manure. As a result, less organic matter decomposition occurred resulting in reduced nitrogen/humus supply for the microbes (BIRKHOFFER et al. 2008).

Based on scientific data, other research groups also found significantly higher values for the pH, organic carbon content, total nitrogen and nitrogen forms in the VHO soil of Nyíregyháza compared to HK (DEMETER et al. 2018). It has been confirmed that organic farming has a positive effect on soil quality and fertility, thereby indirectly affecting soil microbial activity and community. In both seasons, total nitrogen, humus and carbon values show a declining trend from clayey soils to loam sandy.

The NPK supply boundaries of the soils were determined on the basis of the MÉM NAK (1979). Compared to this, VHO is characterized by low nitrogen supply and HK by medium. In terms of phosphorus supply, the AVK, VO, VK and HK areas have a very good supply, the VHO soil has a good classification, while the AO has a medium level. Regarding the potassium supply of the examined soils, I found that in both seasons VO and VK (spring only) are over-supplied; while medium is characterized by AO.

Microbiological results show the positive effect of organic farming on soil microbes. Organic spore-forming microbes are capable of adapting to adverse environmental conditions, with low spore counts in both areas. However, filamentous fungi already play an important role in the breakdown of complex organic matter. Their CFU were significantly higher in organic areas compared to conventional ones, while actinomycetes play a key role in the humification process. Most of the low CFU occurred in acidic sandy soil, probably due to the inhibitory effect of low pH, due to reduced microbial activity (SAHOO et al. 2010). However, in terms of value, the number of CFU grown from spring sampling is higher than that of autumn (since microbes from autumn sampling are characterized by a dormant state, whereas spring germs are characterized by a more active stage).

Organic farming had a significant ( $p \leq 0,05$ ) increase in CFU and activity (FDA, respiration) on the loam soils, and a larger and more active microbial community can contribute to the maintenance of ecological balance as a determinant of soil.

As for respiratory values the BRESP showed values lower than SIR for all soils tested. The metabolic potential of the soil bacterial community in the examined rhizosphere samples was

significantly activated by the addition of glucose. In general, spring values were significantly higher for both BRESP and SIR, except for AO soils, where autumn SIR values were significantly higher than spring. The relative values of the BRESP and SIR values are 4 to 12-fold relative to the SIR.

The total catabolic activity obtained in the microrespiration assay indicates that citrate was the highest of the 23 substrates tested in both seasons, where there was a significant difference only for soil types. Significant differences were found for the following substrates: for the seasons (L-arginine, 3,4-dihydroxy benzoic acid, myo-inositol, D-mannose), soil types/management methods and between season interaction (D-glucose, D-mannose, Na-succinate) and soil types/management methods (L-alanine, L-arginine, L-asparagine-monohydrate, 3,4-dihydroxy benzoic acid, D-fructose).

Consequently, the SIR technique proved to be more efficient, however the MicroResp™ technique provides a more comprehensive view of the activity and pattern of the microbial community based on 23 substrate-induced activity. The diversity of substrates offers greater potential for members of the microbial community in terms of potential nutrients.

I found significantly higher values ( $p \leq 0,05$ ) in organic management methods than in conventional at the main soil chemical and microbiological parameters: total N (%), humus content (%), AL-Ca content (mg/kg), FDA ( $\mu\text{gFl/g soil/hour}$ ), CFU - filiform fungi, spore forming bacterium ( $\log\text{CFU/g dry soil}$ ), SIR-content ( $\mu\text{g CO}_2\text{-C/g soil/hour}$ ), MicroResp™ ( $\mu\text{g CO}_2\text{-C/g soil/hour}$ ) and the major and meso elements (Mg, B, Cu, Zn (mg/kg)). I proved that organic management method had a positive effect on soil quality and fertility, thus indirectly on soil microbial activity and community.

Confirming the scientific data with my results I proved that organic farming has a positive effect on soil quality and fertility, thus on soil microbial activity and community creators. Regarding the tendency in both seasons I found higher values in the Karcag clay (organic), clay-loam (conventional) and Martonvásár loam soil (organic and conventional), compared to the Nyíregyháza sandy loam (organic) and sandy soil (conventional). My results confirmed the fact that organic farming (spring) is more sustainable than conventional treatment. Higher microbial activity detected here provides better soil quality, more efficient nutrient cycling and better growth of crop plants (GE et al. 2013, CREAMER et al. 2016). KÁTAI (2011) Organic areas are characterized by higher microbial biomass (MARTÍNEZ-GARCÍA et al. 2018) and more intense soil respiration. The increased enzyme activity and ATP content may explain the lack of phosphorus deficiency in organic farming despite the low uptake of phosphorus, since these soils have a higher microbiologically bound phosphorus stock, thus increasing their mineralization potential of organic phosphorus.

Overall, microbiological assays based on functional measurement provide a good indication of changes in farming behavior caused by soil microbiota. The results of functional tests show greater sensitivity to the effects of the farming method than the parameters of the microbiota community composition. Among the functional analyzes, the indications that comprehensively characterize the functioning of the microbial community have greater indication sensitivity for example: FDA (bacteria, fungi, algae), MicroResp™ – the catabolic activity of 23 substrates (sugars, amino acids and proteins) – enabling the induction of the function of a larger number of microbial taxa.

Microbiological measurements can not detect community-level transitions within the microbial community. This also confirms the need for the introduction and development of previously described molecular assays.

Adapting the methodology of soil DNA extraction and its microbial community analysis to specific ecological conditions is essential to underpin the research background of organic farming. With the multicriteria methodological development I improved the DNA isolation from soil and the quantitative and qualitative parameters of the obtained nucleic acid. According to my

results, the sample isolated from loam soil (organic management method, spring sampling) gave the strongest nucleic acid yield (DNA concentration:  $34,3 \pm 11,1$  ng/ $\mu$ l; protein content:  $1,11 \pm 0,14$  260/280 nm, (ref.:> 1,8; 260/280 nm); humic acid content:  $0,663 \pm 0,096$  260/230 nm (ref.:> 2; 260/230 nm)) compared to other soil types ( $p \leq 0,05$ ).

I analyzed the above soil nucleic acid indices with a complex evaluation (sum of SRD-rank differences) taking into account several aspects at the same time in the combination of soil type/shaker/shaking time. Vortex shaking for 10 minutes proved to be most effective in organic clay and sandy loam soils.

In the above area, I showed that the genetic community fingerprint is divided into different groups according to soil type and farming method. Sequencing the DNA isolated from soil samples,  $\alpha$ -proteobacteria (*Pseudovibrio denitrificans* (loam soil, organic), *Devosia* sp., *Ancylobacter rudongensis* (loam soil, conventional), *Mesorhizobium* sp. (sandy loam, organic), *Rhizobium* sp. (conventional sand soil) species were detected which are the key indicators of the nitrogen cycle. They play an important role in organic decomposition and nitrogen mineralization. Nitrogen fixing bacteria was successfully detected by the presence of *nifH* gene. There was no significant difference between the diversity indices, however the highest diversity index was showed by the sand soil (conventional) (Nyíregyháza) ( $H'=2,82$ ;  $E=0,89$ ;  $R=23$ ) and the loamy-sandy soil (organic) (Nyíregyháza) ( $H'=2,78$ ;  $E=0,86$ ;  $R=25$ ). In contrast, the smallest diversity was obtained by the loam soil (organic) (Karcag) ( $H'=2,46$ ;  $E=0,87$ ;  $R=17$ ) which however did not show significant differences from other soil types.

My results draws attention to the fact that the above-mentioned multicriteria analysis is essential for understanding the role of soil microbial communities of the rhizosphere and it is necessary to understand the sustainability of this management method. These statements may provide important reference data for the assessment of soil quality, soil fertility and degradation processes. Furthermore the multicriteria analysis also highlights that there may be significant differences in the sensitivity of the indicators used to characterize the microbial community of soil. Selection of indicators with proper sensitivity is essential to detect microbial changes in different soil types. Among the functional studies, the MicroResp<sup>TM</sup> method – according to my knowledge this method is applied only by the Institute of Soil Science and Agrochemistry - is suitable for separating organic and conventional areas based on the pattern of degradation activity of microbial communities.

I would like to draw the attention of the Hungarian Soil Protection Information and Monitoring System (TIM) to consider expanding the scope of microbial studies to include indicators specific to key indicators and functional diversity testing.

I suggest extending the CO<sub>2</sub> production measurement used by TIM with MicroResp<sup>TM</sup> with the following parameters:

- Sampling: in the spring season (due to the activity of soil bacteria)
- 0-25 cm soil depth (rhizosphere examination)
- For organic and conventional farming, monitoring every 3 years would be sufficient to detect the impact of the farming on the microbial community

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## 7. PUBLICATIONS CONNECTED WITH THE TOPIC OF THE THESIS

### Journal articles with IF:

1. **GAZDAG, O.**, TAKÁCS, T., KÖDÖBÖCZ, L., KRETT, G., SZILI-KOVÁCS, T. (2019a): *Alphaproteobacteria* communities depend more on soil types than land managements. *Acta Agriculturae Scandinavica, Section B – Soil & Plant Science*, 69 (2) 147–154. p.  
doi: 10.1080/09064710.2018.1520289  
**IF=0,81** (2018)
2. **GAZDAG, O.**, KOVÁCS, R., PARÁDI, I., FÜZY, A., KÖDÖBÖCZ, L., MUCSI, M., SZILI-KOVÁCS T., INUBUSHI, K., TAKÁCS, T. (2019b): Density and diversity of microbial symbionts under organic and conventional agricultural management. *Microbes and Environments*, Published online: June 13, 2019.  
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### Peer-reviewed journal (MTMT2 list) publications in English:

1. **GAZDAG, O.**, KÖDÖBÖCZ, L., SZILI-KOVÁCS, T., MURÁNYI, A. (2015): Characterization of the efficiency of soybean inoculation. *Agrokémia és Talajtan*, 64 (2) 453–465. p.  
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2. **GAZDAG, O.**, TAKÁCS, T., KÖDÖBÖCZ, L., MUCSI, M., SZILI-KOVÁCS, T. 2018. Soil metabolic activity profiles of the organic and conventional land use at Martonvásár. *Columella: Journal of Agricultural and Environmental Sciences*, 5 (1) 27–35. p.  
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#### **Peer-reviewed journal (MTMT2 list) publications in Hungarian:**

1. MUCSI, M., CSONTOS, P., BORSODI, A., KRETT G., **GAZDAG O.**, SZILI-KOVÁCS, T. (2017): A mikrorespirációs (MikroResp<sup>TM</sup>) módszer alkalmazása apajpusztai szikes talajok mikrobaközösségeinek katabolikus aktivitás mintázatának vizsgálatára. *Agrokémia és Talajtan*, 66 (1) 165–179. p.  
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3. **GAZDAG, O.**, TAKÁCS, T., KÖDÖBÖCZ, L., MUCSI, M., INUBUSHI, K., SZILI-KOVÁCS, T. (2018): Comparing organic and conventional farming systems at three different soils by physiological profiles of the soil microbial assemblage. In: 32<sup>nd</sup> Annual Meeting of Japanese Society for Microbial Ecology & 10<sup>th</sup> Asian Symposium on Microbial Ecology: Book of Abstracts. Konferencia helye, ideje: Okinawa, Japán, 2018.07.11-2018.07.13. Okinawa: ASME; JSME; SFEN, 409. p. 1 p.

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