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Tsedekech Gebremeskel Weldmichael

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CHARACTERIZATION AND EVALUATION OF SOME MAJOR SOIL GROUPS OF HUNGARY AND ETHIOPIA BY SELECTED BIOLOGICAL AND PHYSICOCHEMICAL METHODS

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TSEDEKECH GEBREMESKEL WELDMICHAEL

GÖDÖLLŐ

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Name of PhD School:	Doctoral School of Environmental Sciences
Discipline:	Environmental Sciences
Head:	Csákiné Dr. Michéli Erika, CMHAS MATE, Institute of Environmental Sciences, Department of Soil Science
Supervisor (s):	Csákiné Dr. Michéli Erika, CMHAS MATE, Institute of Environmental Sciences, Department of Soil Science Dr. Barbara Simon, PhD MATE, Institute of Environmental Sciences, Department of Soil Science

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Approval of Head of Doctoral School Approval of Supervisor(s)

1. INTRODUCTION AND OBJECTIVES

Soil biodiversity is recognized as a crucial player in guaranteeing the functioning of soil and as a provider of several ecosystem services (Orgiazzi *et al*., 2016). These include nutrient cycling, soil formation, primary production, water quality and quantity, control pest and disease incidence in the agricultural and natural ecosystem, and human diseases (Turbé *et al*., 2010). Despite the broad range of ecosystem services soil biodiversity provides, it has been under constant threats mainly due to human activity. At present, biodiversity loss has become a global challenge as numerous researches showed that it will negatively affect ecosystem services on which society depends (Vandewalle *et al.*, 2010).

Currently, the significance of soil biota for the improvement of soil fertility through biological processes becomes a key component of a strategy towards sustainable soil management. Earthworm and microbial communities are important bioindicators to monitor soil fertility because of the vital roles they play in ecosystem functioning (Stone *et al.*, 2016). Nevertheless, these indicators have rarely been studied together across a range of soil and land use types (LUTs). Understanding the effects of LUT and soil types on soil biodiversity has paramount importance to monitor the responses of soil ecosystem to global change and to design effective sustainable soil management systems. The objectives of this study are:

- To examine patterns of earthworm (abundance, biomass, and species richness) and SMR in relation to soil and LUTs in some major soil groups of Hungary.
- To describe the bacterial community structure and SMR of some major soil groups of Ethiopia.

- To identify important plant growth promoting bacteria (PGPB) in major agricultural soils of Ethiopia.
- To establish relationship between the biological parameters and the soil physicochemical properties.
- To identify key edaphic factors linked to the variability of SMR and earthworm communities in Hungary.
- To distinguish major soil properties that influence the SMR and bacterial communities in major agricultural soils of Ethiopia.
- To evaluate how the LUT and soil properties affect the earthworm (abundance, biomass, and species richness) and SMR of some major soil groups of Hungary, and SMR and bacterial communities of some major soil groups of Ethiopia.

2. MATERIALS AND METHODS

2.1. Site description

Hungary

The study was carried out on the experimental farm of Hungarian University of Agriculture and Life Sciences at Józsefmajor nearby Hatvan (N 47° 40′ 5", E 19° 40′ 11"), Hort (N 47º 41' 36.90", E 19º 48' 53.04"; N 47º 41'42.03", E 19º48' 50.46"), Verpelét (N 47° 86' 91.62", E 20° 19' 99.45") in Heves County; Gödöllő hill and Szárítópuszta in Gödöllő town (N 47° 35' 47.65", E 19° 21' 18.54"), Pest County; Szárhalmi forest (N 47° 41' 41", E 16° 50' 31"), Károly-magaslat (N 47° 39' 49.14", E 16° 33' 41.10") in Győr -Moson-Sopron County; Csobánc (N 46° 52' 18.50", E 17° 30' 16.35") in Veszprém County (Figure 1).

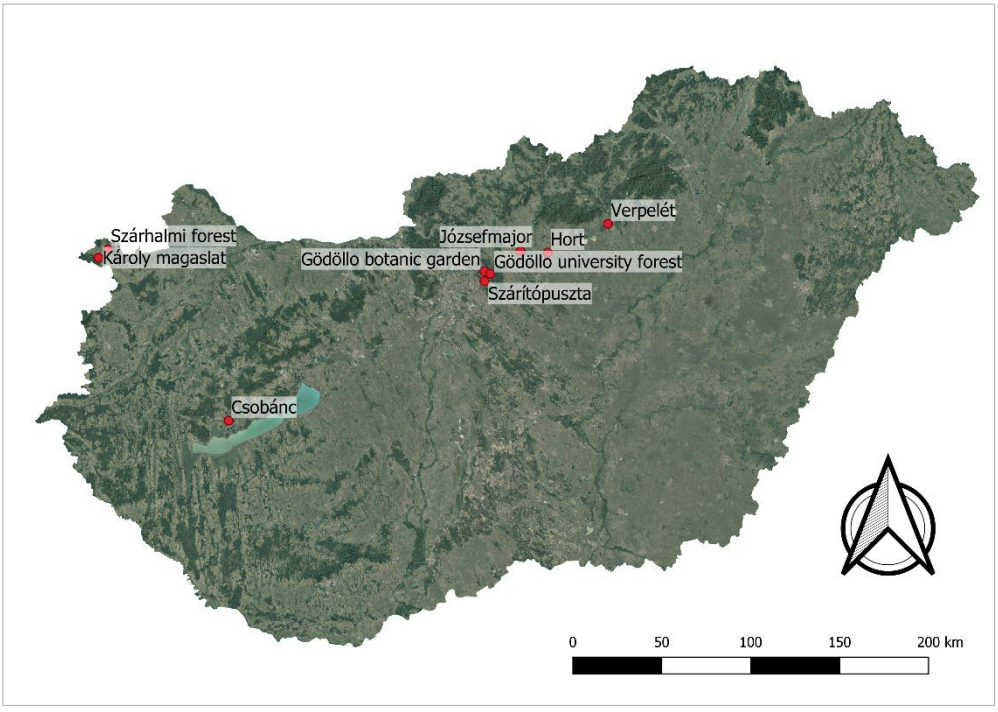

Figure 1. Location map showing study sites in Hungary

The experimental farm of Józsefmajor, Hort, and Verpelét are part of 'North Plain Alluvial Fan' which is a small geographical area of the North Hungarian Mountain. The average annual temperature ranges 10–11^{o}C and the mean rainfall is between 550–600 mm. Luvisols and Chernozems are the dominant soil types in the area. The elevation of the region varies between 128 and 350 m. The mean annual precipitation ranges from 580–610 mm and the mean annual temperature is 9.5–10^{o}C (Dövényi *et al*., 2008).

The Gödöllő sites belong to the Gödöllő-Monori hilly region which is part of Northern Hungarian Mountain. The most common reference soil groups (RSGs) in the region are Luvisols, Cambisols, Arenosols, and Chernozems. The mean annual temperature ranges from 9.5–10^{o}C and the annual precipitation is about 600 mm.

Szárhalmi forest and Károly-magaslat sites are located in the north-western part of Hungary at the western border of the country. The mean annual temperature and precipitation are 9.4^{o}C and 727 mm, respectively. The characteristic soil types are Fluvisols in the downtown area while Umbrisols (acidic, non-podzolic brown forest soils), Cambisols (brown earths), and Gleysols (meadow soils) are typical in the area of Sopron Hills and on peri-urban suburb (IUSS Working Group WRB, 2015; Michéli *et al*., 2006). Csobánc is a 376 m high hill in the Tapolca Basin in Western Hungary near Lake Balaton. Mean annual temperature and precipitation are10.2^{o}C and 716 mm, respectively.

Ethiopia

The sampling sites located in Laelay Maichew (38° 12' 19" E, 13° 55' 53" N) and Atsbi Wenberta districts (39° 30'-39° 45' E, 13° 30'- 13° 45' N) situated in central and eastern part of Tigray Regional State, Ethiopia (Figure 2).

Laelay Maichew district is agro-ecologically classified in the semiarid region characterized by short rainy period. The altitude varies between 1842 and 2250 m. The average annual rainfall ranges between 550–750 mm and the mean minimum and maximum temperatures are 11.7˚C and 26.1˚C, respectively (Kahsay and Mulugeta, 2014). The main RSGs are Cambisols on undulating plains and rolling landforms; Leptosols on hilly and steep to very steep lands and Vertisols are found on the flat plateau plains (Brhane and Mekonen, 2009).

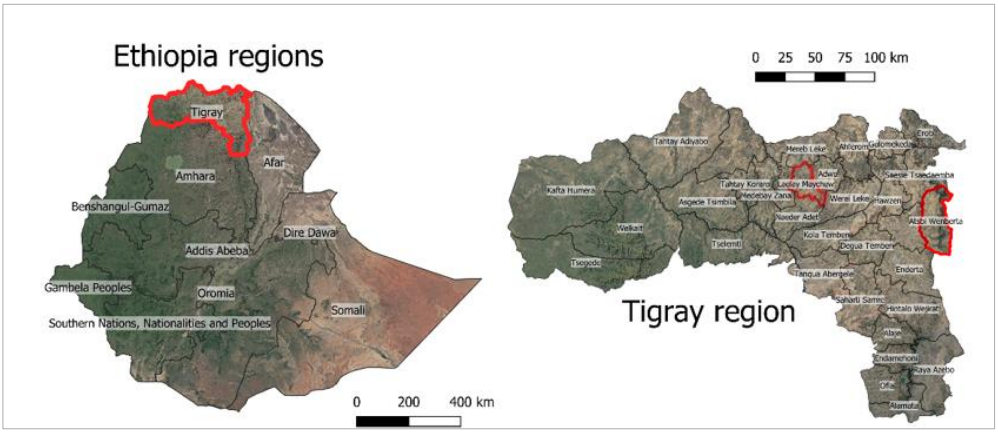

Figure 2. Location map showing Tigray region within Ethiopia and Laelay Maichew and Atsbi Wenberta districts within Tigray region

The elevation of Atsbi Wenberta district varies from 918 to 3069 m. 75% of the district is upper highlands (2600 m or above) and only 25% is found in midlands (between 1500 and 2600 m) and lowlands (below 1500 m). The district falls in sub-tropical agro-climatic zone and has an average daily temperature between 15°C and 30°C and the mean annual precipitation rate is about 529 mm. Lithic Leptosols is a predominant soil type in the area (Gebremedhin, 2004).

2.2. Soil sampling

Soil samples from Józsefmajor (JM1, JM2, JM3), Gödöllő hill (GUF, GBG), Szárítópuszta (SZP1, SZP2), Hort (HOGR, HOAR), and Verpelét (VERP) sites were taken from the upper 25 cm of the soil. However, soil samples from Szárhalmi forest (SZHE), Csobánc (CSOB), and Károly-magaslat (KAMG) sites were collected along the two depths of the soil (0–10 cm and 10–25 cm). Samples were collected on 1m x 1m plots, three meters away from the main soil profile, in three different direction as shown in Figure 3. From each plot, one bulk soil sample, roughly measured 1 kg and three undisturbed soil cores for bulk density analysis were taken randomly using spade and volumetric core, respectively. Samples from each soil profile and/or the same depth (1–10 or 10–25 for Szárhalmi forest, Csobánc, and Károly-magaslat sites) compiled together and mixed thoroughly, divided into two subsamples, one portion for SMR and the other for physicochemical analyses.

In Ethiopia, soil samples were collected from four agricultural fields (LMH-1, LMH-2, LMH-10, ATS-3) that have been under cultivation of teff (*Eragrostis tef (Zuccagni) Trotter*) and wheat (*TritiFcum aestivum L*). The sampling sites represented by four common RSGs in agricultural landscapes of Ethiopia i.e., Nitisols, Vertisols, Cambisols, and Luvisols. At each site, eight points around the main soil profile in a 10 m radius were designated as sampling points (Figure 4). Soil samples (0–25 cm depth) were collected from these points, compiled, and mixed thoroughly to make a composite sample. From the composite sample, three subsamples were taken for: a) physiochemical, b) SMR, c) soil bacterial genomic analyses. Soils for SMR and bacterial genomic stored in refrigerator at 4^{o}C. However, soils for physicochemical analyses were air dried and sieved through 2mm mesh and stored in room temperature until the analyses.

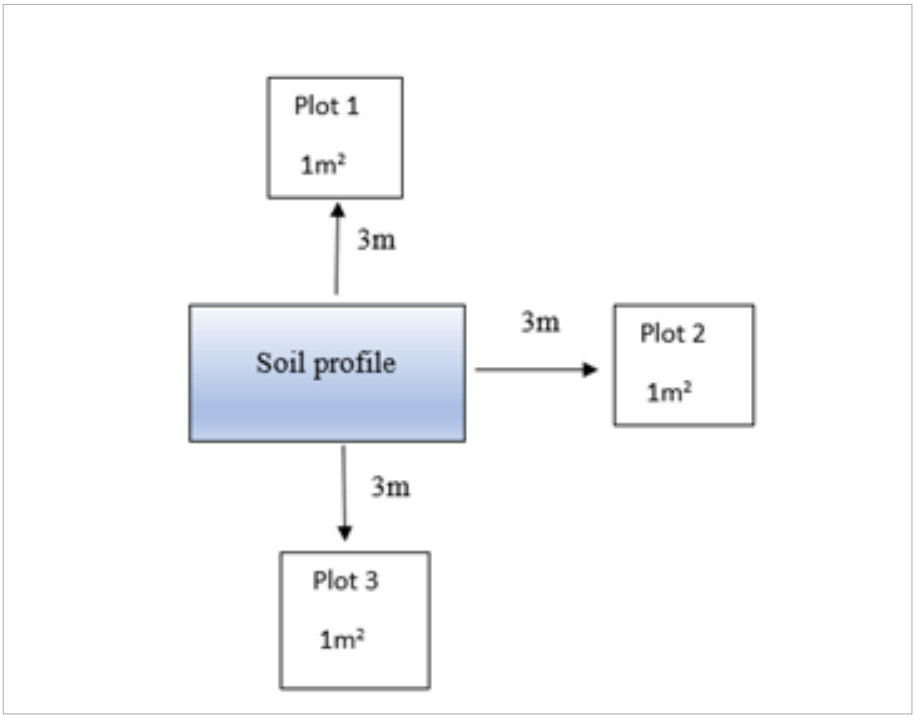

Figure 3. Soil sampling scheme used in Hungary

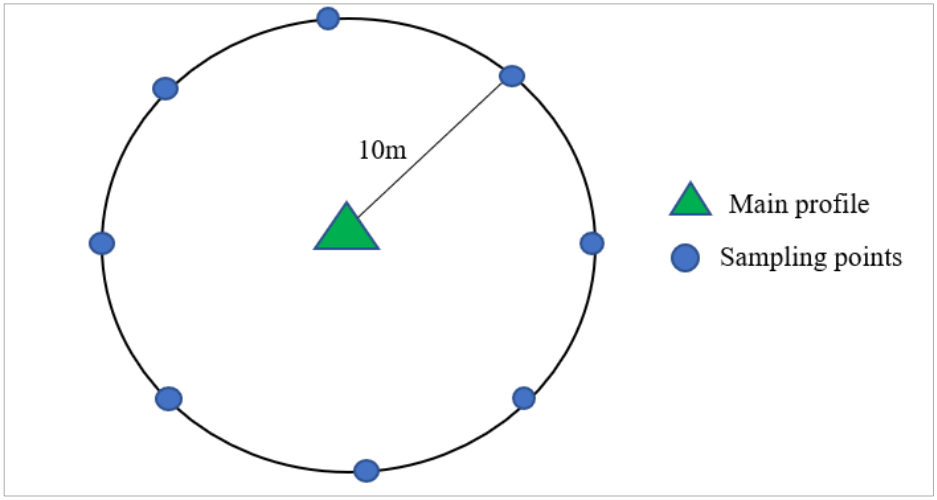

Figure 4. Sampling scheme used in Ethiopia

2.3. Earthworm extraction and soil laboratory measurements

2.3.1. Physicochemical soil analyses

Soil pH measured potentiometrically in the supernatant suspension of a 1:2.5 soil: liquid (H_2O) mixture (Buzás 1988). Bulk density and moisture content (MC) were determined by gravimetric method (Buzás 1993). Available nitrogen ($NH4^+$-N and NO_3^--N) was measured using Parnas-Wagner Apparatus (Egnér *et al*., 1969). Available potassium and phosphorus were estimated based on ammonium-lactate solution method (AL method) (Egnér *et al*., 1960). Soil organic carbon (SOC) was measured using Walkley-Black method (Walkley and Black, 1934). $CaCO_3$ content was determined using Scheibler calcimeter (Buzás, 1988). Available Ca^{2+}, Mg^{2+}, and Na^+ were extracted in 1N KCl, determined by EDTA titration (Egnér *et al*., 1960). Cation exchange capacity (CEC) and exchangeable basic cations (Ca^{2+}, Mg^{2+}, K^+, and Na^+) following Mehlich 3 extraction (Mehlich, 1953). Humic substance (E_4/E_6, ratio of the absorbances at 465 nm and at 665 nm) was determined using spectrometer (Page *et al*., 1982).

2.3.2. Bacterial genomic analysis

DNA isolation and purification

For extracting the total DNA, the method of Högfors-Rönnholm *et al.* (2018) was carried out modified with sonication on ice for one minute and break the slurry in 50 ml falcon tubes for one minute in three rounds after vortexing. DNA was extracted from the collected supernatant using Quick-Dna Soil Microbe Miniprep Kit (Zymo Research, USA) according to the manufacturer's instruction. DNA was visualized by gel electrophoresis. Further, the quality and the integrity of isolated DNA was determined by

Nanodrop spectrophotometer ND 2000 (Nano-Drop Technologies, Wilmington, DE, USA).

16S rDNA amplicon sequencing and data handling

To assess the bacterial community composition of the soils sample precisely, Illumina 16S rDNA amplicon sequencing was carried out. The variable V3 and V4 region of the 16S rDNA was amplified by using the primers recommended by Klindworth *et al.* (2013), 16S amplicon PCR forward (5′-**TCGT CGGCAGCGTCAGATGTG TATAAGAGACAG**CCTA CGGGNGGCWGCAG-3′) named 16S Amplicon PCR Forward Primer-S-D-Bact-0341-b-S-17-N and reverse (5′-**GTCT CGTGGGCT CGGAGATGTGTATAAGAGACG**GACTACHVGGGTATCTAATCC-3′), named 16S Amplicon PCR Reverse Primer-S-D-Bact-0785-a-A-21-N primers with Illumina adapter overhanging nucleotide sequences written in bold. PCR reaction mixture in a final volume of 50 µl that contained 12.5 ng of DNA, 0.2 µM of each Illumina 16S primers and 12.5 µl of 2X KAPA HiFi Environ Sci Pollut Res HotStart Ready Mix (KAPABiosystems, London, United Kingdom). The temperature profile used was an initial denaturation for 3 min at 95°C, followed by 25 cycles of denaturation for 0.5 min at 95°C, annealing for 0.5 min at 55°C and elongation for 0.5 min at 72°C. The last step was a final extension for 5 min at 72°C. All amplifications were carried out in a ProFlex PCR System (Applied Biosystems by Life Technologies, USA). Amplicons were analysed under UV light after electrophoresis in 1% (w/v) agarose gel stained with EtBr. Paired-end fragment reads were generated on an Illumina MiSeq sequencer using MiSeq Reagent Kit v3 (600-cycle). Read numbers were the following: 77065 for LMH-1 for sample, 60265 for LMH-10 and 67937 for LMH-2 and 58532 for ATS-3 sample (BF). Primary data analysis (base-calling) was carried out with Bbcl2fastq^ software (v2.17.1.14, Illumina). Reads were quality and length trimmed in CLC

Genomics Workbench Tool 9.5.1 using an error probability of 0.05 (Q13) and a minimum length of 50 nucleotides as a threshold. Trimmed sequences were processed using mothur v1.35 as recommended by the MiSeq SOP page (http://www.mothur.org/wiki/MiSeq_SOP downloaded at 22/06/2019) (Kozich *et al.*, 2013). Sequences were assorted based on the alignment using SILVA 132 SSURef NR99 database (Quast *et al.*, 2013). Chimera detection was performed with mothur's uchime command (Edgar *et al.*, 2011), and 'split.abund' command was also used to remove singleton reads according to (Kunin *et al.*, 2010). After all quality control, 38132 reads/sample (400 bp/read) were taxonomically investigated. Taxonomic assignments were made against SILVA release 132 applying a minimum bootstrap confidence score of 80%. Operational taxonomic units (OTUs) were assigned at 97% similarity threshold level as suggested by Tindall *et al.* (2010). For prokaryotic species delineation. Raw sequence reads were deposited in NCBI SRA under BioProject ID SAMN14390016, SAMN14390017, SAMN14390018, SAMN14390019.

2.3.3. Soil microbial respiration

The analysis of microbial soil respiration (MR) followed ISO 16072:2002(E) and Cheng *et al*., (2013) guideline with minor modification. Approximately 50 gm fresh soil was placed in airtight jar and 10 ml deionized water was added to adjust moisture content. A conical containing 10 ml 1.0 M NaOH was placed in same jar and the samples were incubated for 10 days in dark at room temperature (22^0C). After 10 days, the conical was removed and 1 ml $BaCl_2$ was added in the NaOH solution to precipitate trapped CO_2. The determination was carried out in triplicates. Controls (triplicate flasks without soil) were also prepared.

2.3.4. Earthworms (abundance, total biomass, and species richness)

The extraction of earthworms was done by using hand sorting method as described by ISO 23611-1 (2006) guideline. According to the pattern shown in Figure 3., from each (1m x 1m) plot, 25 × 25 × 25 cm soil blocks were taken using spade. The excavated soil was spread on the plastic sheet, total abundance (individual m^{-2}) and fresh biomass (g m^{-2}) were estimated. The species richness was performed using identification key found in Csuzdi and Zicsi (2003).

2.4. Statistical analyses

Statistical analyses were performed in R software (R Development Core Team, 2017). All data sets were tested for normality and the equality of group variances using Shapiro-Wilk normality and Levene's tests, respectively. One-way Analysis of Variance (ANOVA) for parametric data or Kruskal-Wallis test for non-parametric data were performed to compare variability of soil properties among sites and LUTs. Tukey's HSD post hoc test ($p < 0.05$) was used for multiple comparisons of means of soil properties across sites and LUTs. Pearson's correlation and principal component analysis (PCA) were employed to examine the relationship between various soil parameters (correlation was assumed significant when $p < 0.05$). Independent samples t-test was used to compare soil parameters across two soil depths and diagnostic horizons. To assess the relative abundance of bacterial community and visualized the hierarchical nature of taxonomic classifications, a heat tree, according to the 38132 reads (400bp)/sample was created for those OTUs showing relative abundance over 1% by using Metacoder R package (Foster *et al*., 2017). Graph for SMR measured at two soil depths and monoplot showing the relationship of soil parameters were performed using Analyse-it for Microsoft Excel (version 2.20).

3. RESULTS AND DISCUSSIONS

3.1. Effects of soil and LUT on earthworm communities and soil microbial respiration

This section presents the results of soil samples taken from Józsefmajor, Szárítópuszta, and Gödöllő sites in 2017. Soils were described, classified, and grouped into mollic (Chernozems and Phaeozems) and non-mollic RSGs (Luvisols and Arenosol) categories based on the presence/absence of mollic diagnostic horizon.

3.1.1. Variation of SMR in relation to soil and LUT

SMR was significantly higher in mollic soils compared to non-mollic soils ($p < 0.01$). Available Ca^{2+} ($r = 0.80$), MC ($r = 0.72$), and available Mg^{2+} ($r = 0.69$) were found to be strongly correlated with SMR. The result of PCA showed that 36% and 25% of the total variance across sites were explained by PC1 and PC2, respectively. PC1 clearly separated mollic soils from non-mollic soils mainly based on Ca^{2+} (Figure 5). There was a negative correlation between available nitrogen (NO_3^--N and NH_4^+-N) and microbial respiration.

The mean basal respiration did not show significant difference between land-use types (LUTs) within mollic diagnostic category ($p < 0.05$), but it differed within non-mollic category ($p < 0.05$) (Figure 6). This study found a significant variation of SMR among soils of different textures ($p < 0.001$), with the highest value in silt clay loam (SiCL) soils and lowest in sand (S) soils.

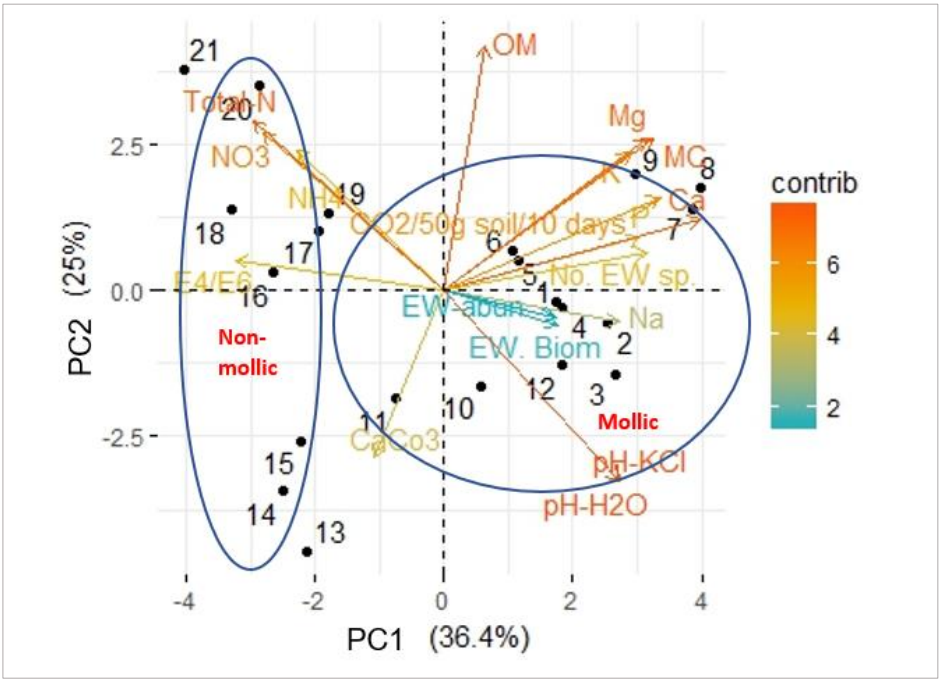

Figure 5. Principal component analysis showing the variance in soil properties across sites. Sites are coded in number (1-12 mollic soils and 13-21 non-mollic soils). Red color indicates high level of contribution whereas the blue color implies low contribution to the total variation.

3.1.2. Pattern of earthworm communities across soil and LUTs

Earthworm abundance and biomass were significantly higher in mollic soils, compared to non-mollic soils ($P < 0.01$) and greater in grassland than arable land in mollic soils and forest compared to arable land in non-mollic soils. A total of five earthworm species were identified. The grassy Chernozem (JM3) soils had the highest number of earthworm species (4 out of 5). *Aporrectodea caliginosa* (Savigny, 1826) was the most abundant earthworm species in the study area. This study did not find a clear association of earthworms (abundance, biomass) and other soil properties.

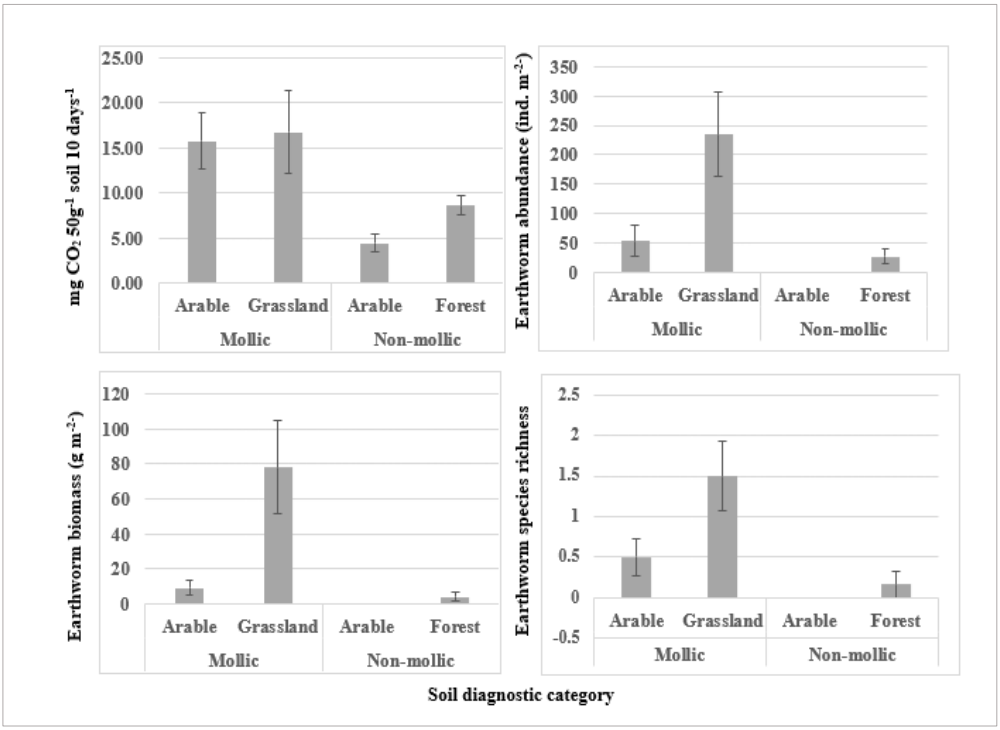

Figure 6. Effect of soil and LUT on SMR and earthworm (abundance, biomass, and species richness) (mean of three measurements and upper and lower error bars showing confidence interval).

3.2. Influence of depth on SMR and major soil chemical attributes that determine its variability

The following results and discussions are based on experiment done in November 2018 at Szárhalmi forest (SZHE), Károly-magaslat (KAMG), and Csobánc (CSOB) sites. In this study, soil samples were collected at two depths of the soil (0–10; 10–15 cm) and important soil chemical properties and SMR were analyzed.

3.2.1. Soil depth and soil chemical properties

The SOM was higher in surface than subsurface soils and ranged between 3.63 to 39.53% in surface and from 3.6 to 10.38% in subsurface layer, respectively.

The study did not find a significant decrement of soil pH with soil depth; however, soil depth had a strong effect on total N (mg kg^{-1}), total N (mg kg^{-1}), NO_3^--N (mg kg^{-1}), and NH_4^+-N (mg kg-1) (p = 0.00004, 0.0005, and 0.02, respectively). In general, all exchangeable base cations showed similar declining patterns in the subsoils from the surface soils while AL-P_2O_5 increasing with depth.

3.2.2. Depth effect on SMR as it influenced by soil chemical properties

SMR was significantly different among the two soil depths (p =0.0005) and decreased with increasing depth (Figure 7).

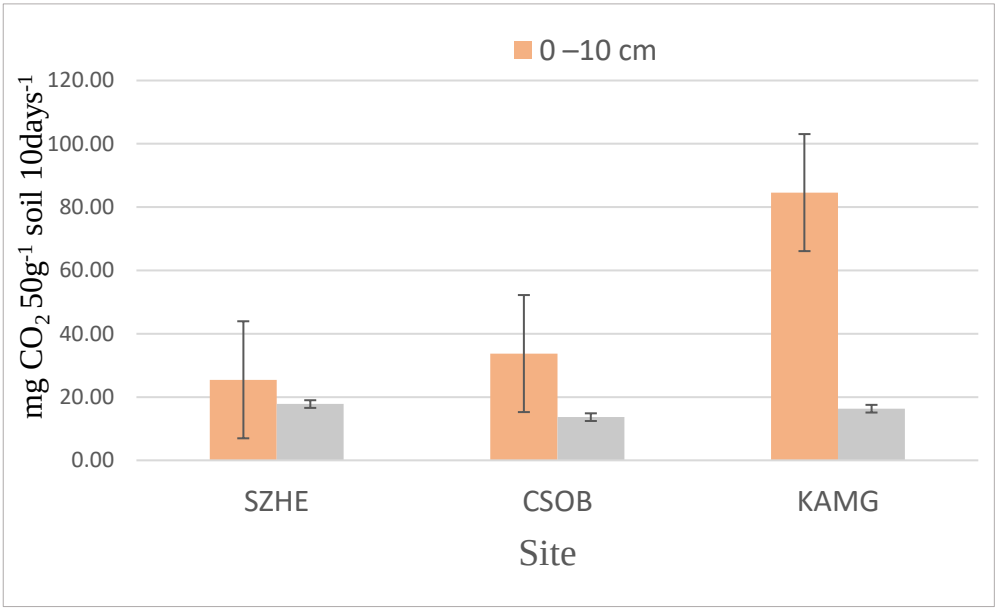

Figure 7. SMR measured at two soil depths across three sites (mean of three measurements and upper and lower error bars showing confidence interval). Szárhalmi forest (SZHE), Károly-magaslat (KAMG), Csobánc (CSOB)

In this study, the variation of SMR was in accordance with the change in available N and SOM along the two soil depths. Accordingly, SMR had a significant positive correlation with total N, NO_3^--N, NH_4^+-N, and SOM, respectively. Further, the PCA shows that PC1explains 51.1% of the total variation, and SOM, CEC, SMR, and available N were the most prominent predictors, respectively. The second PC accounts for 28.6% of the variation, and P_2O_5 was the key contributor (Figure 8).

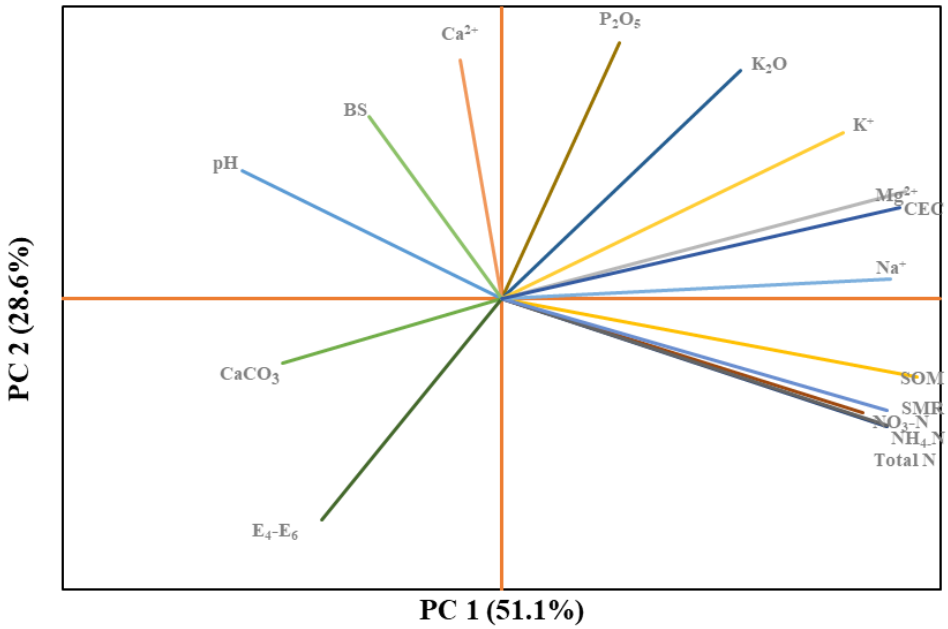

Figure 8. Correlation monoplot showing the relationship between the variables. Small angle between the soil properties represents positively correlated, an angle of 90^o and close to 180^o indicate the variables are not correlated and negatively correlated respectively, and the length of the line and its closeness to the square, represent how well the variable is represented in the plot (short and far line implies poor representation).

3.3. Response of SMR to different LUTs

This section presents and discusses the results of the soil samples collected from Gödöllő, Szárítópuszta, and Hort sites in 2018.

3.3.1. Soil physicochemical properties among LUTs

SOM differed significantly among the LUTs ($p = 0.03$). Soils from the arable land showed significantly lower concentration of SOM with mean of 2.83% compared to soils collected from forest (4.85%) and grassland (5.06%), respectively. Significant differences in available form of N (NH_4^+-N, NO_3^--N, and total N), K_2O were detected between LUT where grassland soils had the highest. K_2O content whereas the available N was recorded highest in forest soils. In comparison with grassland and forest soils, there was a high concentration of $CaCO_3$ and P_2O_5 in arable soils. CEC did not show significant difference among the land used type whereby the highest value recorded in grassland soils. Among the base cations, Ca^{2+} and K^+ were significantly higher in arable soils followed by grassland and forest soils respectively, whereas the amount of Mg^{2+} and Na^+ were highest in grassland and lowest in forest.

3.3.2. Variation of SMR across LUT

SMR was significantly differ among the LUTs ($p < 0.01$). The grassland showed significantly higher SMR compared to the forest and arable land (Figure 9). In this study, SMR was found to be positively correlated with MC ($r = 0.67$; $p < 0.01$). Mg^{2+} ($r = 0.61$; $p < 0.01$), K_2O ($r = 0.58$; $p < 0.05$), and SOC ($r = 0.56$; $p < 0.05$).

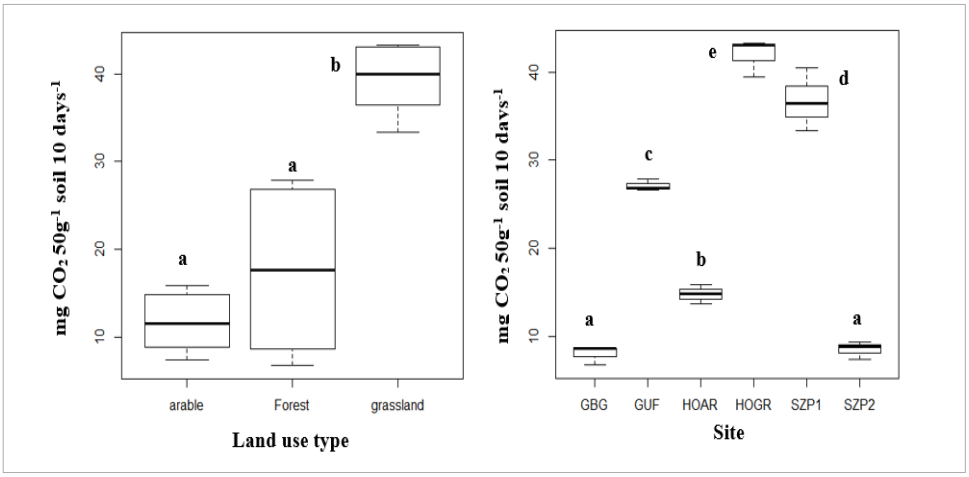

Figure 9. SMR across LUTs (left) and site (right), with standard error bars. Different letters indicate significant differences ($p < 0.05$, n=3(site) n=6. Abbreviations: Hort1 (HOGR), Hort2 (HOAR), Gödöllő botanical garden (GBG), Gödöllő university forest (GUF), Szárítópuszta 1 (SZP1), Szárítópuszta 2 (SZP2)

3.4. Bacterial community structure and soil microbial respiration of selected arable soils of Ethiopia

In this section, the patterns of SMR and bacterial community structure across four common agricultural soils of Ethiopia is discussed.

3.4.1. SMR and key soil physicochemical properties

The rate of SMR ranged from 11.22 to 47.08 mg CO_2 $50g^{-1}$ soil 10 $days^{-1}$, and it was significantly higher in Nitisol with a high content of SOC and P_2O_5. In general, this study highlighted that the variation of SMR in the study area was closely linked with the variation of P_2O_5, SOC, and Mg^{2+}.

3.4.2. Bacteria community structure

Comparing the genus number according to the 400 bp amplicon sequencing results, Nitisol was the richest with 475 genera, Cambisol was the second with 389 genera, Luvisol showed 351 and Vertisol contained the lowest number 315. From these genera's operational taxonomic units (OTUs) (family or order

in some cases), only 31 were showing relative abundance over 1%. The 31 genera showing abundance over 1% covered the 79% of all OTUs of Vertisol, 72% of Luvisol, 66% of Cambisol, and 59% of Nitisol. Based on the heat tree figure, the Luvisol and Cambisol have a slight difference among the identified bacteria genera (Figure 10). The following phyla showed the greatest abundance in the samples: *Actinobacteria, Chloroflexi, Proteobacteria, Acidobacteria, Gemmatimonadetes,* and *Planctomycetes*. The three most abundant phyla (*Actinobacteria*, *Chloroflexi*, *Proteobacteria*) in total comprised 65%, 54%, 51%, and 46% of the total bacterial abundance in Vertisol, Luvisol, Nitisol, and Cambisol, respectively.

The richest phylum, *Actinobacteria*, was represented by the following classes over 1% abundance: *Thermoleophilia, Actinobacteria, Acidimicrobiia,* and *Rubrobacter*. In Vertisol, from the total abundance of phylum *Actinobacteria* (49%), *Thermoleophilia* and *Actinobacteria* accounted for 20% and 18%, respectively. Within *Proteobacteria, Alphaproteobacteria* was the most abundant class, followed by *Betaproteobacteria*. Among the *Chloroflexi* phylum, two classes were abundant over 1%: *Chloroflexi* and an uncultured class, *Bacterium Ellin-6519*. The LMH soils had a greater abundance (over 4%) of *Bacterium Ellin-6519* but its abundance was only 0.84% in the Nitisol.

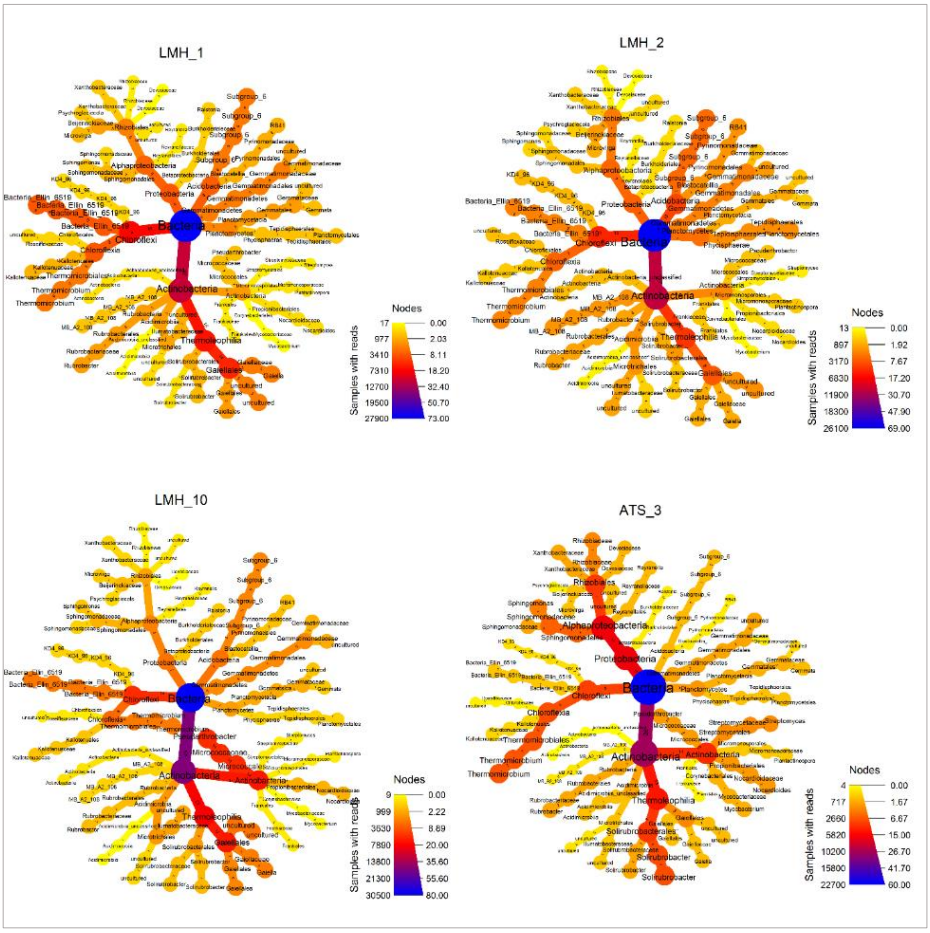

Figure 10. Heat tree map showing relative abundance of bacteria genera within the most abundant bacterial phyla

3.4.3. The relative abundance of dominant agroecosystem bacteria, their determinants, and ecological roles

The most common members of the PGPB are the *Azotobacter*, *Bacillus*, *Pseudomonas*, *Streptomyces* genera, and *Rhizobiaceae* family which are able to promote plant growth with different enzymatic activities such as N fixation, P mobilization, Indole Acetic Acid (IAA), and extracellular polysaccharide (EPS) production. The predominant PGPB in the studied soil belongs to *Proteobacteria*. The *Alphaproteobacteria* was the most dominant class,

occupied the highest abundance in Nitisol. The majority of sequences in the *Alphaproteobacteria* were affiliated with the order *Rhizobiales* (including *Rhizobiaceae*, *Beijerinckiaceae*, *Xanthobacteraceae*, *Devosiaceae* family), which could perform nitrogen fixation, organic matter decomposition, and plant growth promotion (Andrea *et al*., 2017).

Altogether the greatest abundance of the known PGPB was shown by the Nitisol with 15%, represented over 1% abundance by the following taxa: *Streptomyces, Sphingomonas, Ralstonia* genus, *Rhizobiaceae, Frankiaceae, Devosiaceae* family. The other soils had a total of only 4% PGPB abundance from the previously mentioned taxa.

3.5. Comparison of selected bioindicators among similar RSGs of Hungary and Ethiopia

Among the investigated RSG, only Luvisols and Vertisols were found both in Hungary and Ethiopia sites, hence the comparison was only made between these RSGs. The results were based on soils samples collected from GBG (Luvisol), GUF (Luvisol), and VERP (Vertisol) sites (Hungary); LMH-1 (Luvisol) and LMH-10 (Vertisol) (Ethiopia). The earthworms were only collected from sites in Hungary.

3.5.1. Variation of SMR among RSGs

The rate of SMR was relatively higher in both Luvisol and Vertisol of Hungary compared to Luvisol and Vertisol of Ethiopia, although the difference was not big. In both soil samples from Hungary and Ethiopia, SMR recorded higher in Luvisol than Vertisol (Figure 11).

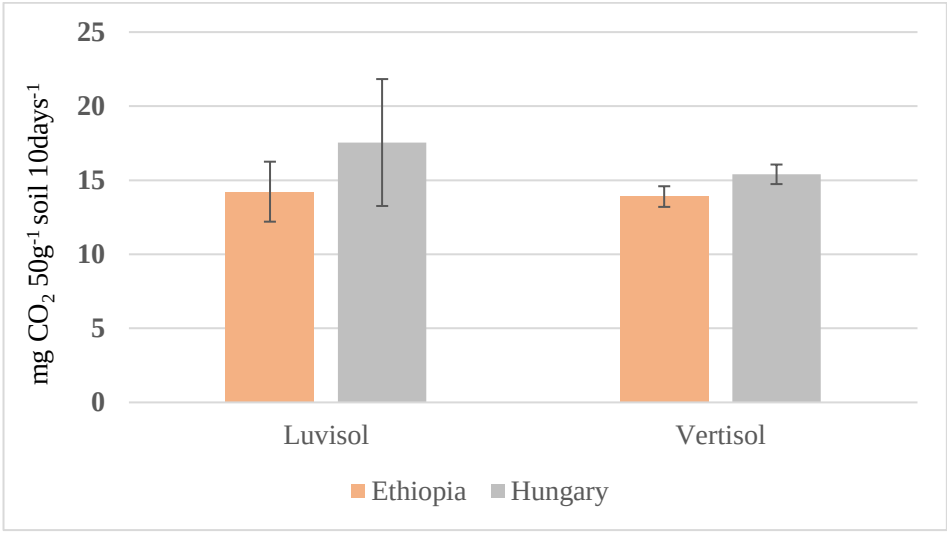

Figure 11. SMR among the two RSGs of Hungary and Ethiopia

3.5.2. Patterns of bacterial community structure across RSGs

At phylum level, *Actinobacteria* highly dominated the soils from Ethiopia compared to the soils from Hungary (Figure 12). In Ethiopia soils, *Actinobacteria* phylum was more represented by *Thermoleophilia* class while in Hungary soils it was dominated by *Actinomycetia* class. Within *Actinobacteria* phylum, genus *Gailla* highly characterized soils of Ethiopia, in Vertisol (17%) and in Luvisol (14%). Its' abundance was low in soils from Hungary, although it dominated the VERP (Vertisol) sites with 4 %.

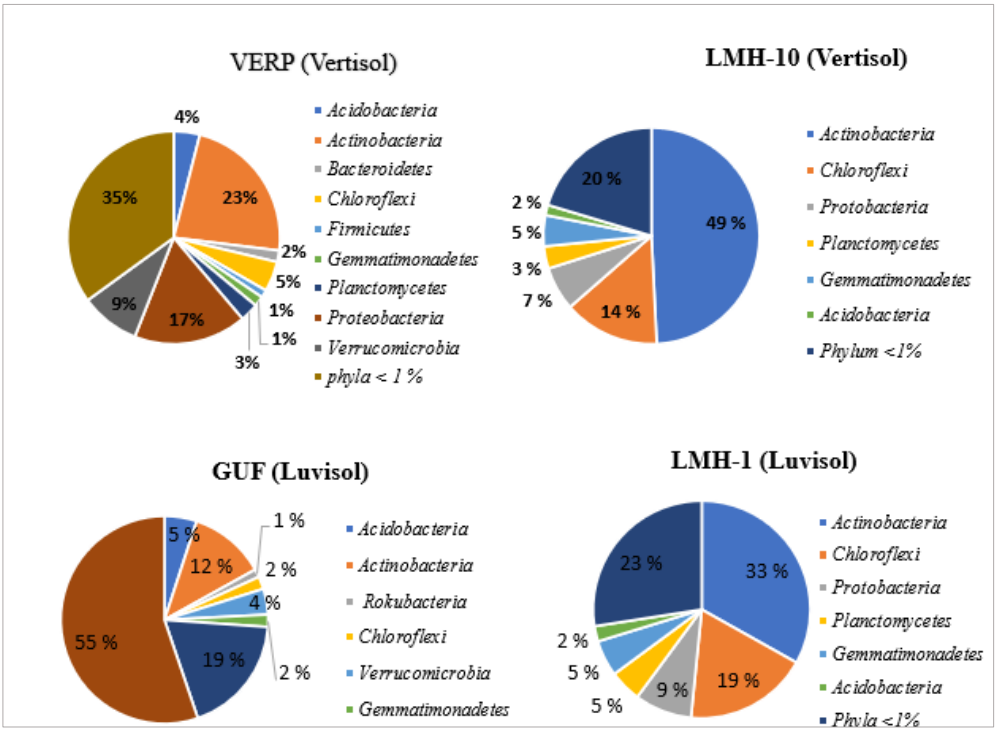

Figure 12. Distribution of bacteria phyla among RSGs of Hungary (left) and Ethiopia (right)

The highest abundance of *Chloroflexi* was found in LMH-1 (18%) followed by LMH-10 (14%), VERP (4%), GUF (2%), respectively. *Chloroflexi* from *bacteria Ellin 6519* and *Thermomicrobium roseum* genus presented in large number in soils from Ethiopia while it formed a minor part of the bacterial communities of samples from Hungary, mainly from representative of *bacteria KD4_96*. *Acidobacteria* phylum was more prevalent in GUF (5%) followed by VERP (4%), and LMH-10 and LMH-1 (2%). The bacterial communities in GUF demonstrated an increase in the proportion of *Proteobacteria* phylum (19%) from the *Bradyrhizobium* genus, followed by VERP (17%) from family *Xanthobacteraceae*. Predominant proportion of *Proteobacteria* in Ethiopia soils were belonging to genus *Siphingomonas* and family *Beijerinckiaceae*.

The distribution of sequences between members of *Acidobacteria* was quite different among samples from Hungary and Ethiopia. The *Acidobacteriaceae* family and *Subgroup_6* mostly characterized soils from Hungary, whereas family *Pyrinomonadaceae* represented the *Acidobacteria* communities of soils from Ethiopia in more than 1% abundance. Phylum *Verrucomicrobia* were only detected in greater than 1 % abundance in samples from Hungary (VERP: 9% and GUF: 4%). Phylum *Verrucomicrobia* were only detected in greater than 1% abundance in samples from Hungary (VERP: 9% and GUF: 4%).

Altogether, this study found the greatest difference in the abundance of *Gailla, Ellin 6519, Thermomicrobium roseum, Gemmatimonadaceae, Acidobacteria Subgroup 6* among the investigated Hungary and Ethiopia soils. The first four bacterial groups found in great abundance in Ethiopia soils and the last in Hungary soils. The Luvisol and Vertisol of Ethiopia greatly differed in *Pseudarthrobacter* abundance, which was higher in Vertisol. The biggest difference among Vertisol and Luvisol of Hungary was with respect to a high occurrence of genus *Canddatus Udasobacter* belonging to *Verrucomicrobia* phylum in Vertisol samples.

3.5.3. Earthworm communities among Vertisols and Luvisols of Hungary

All measured earthworm parameters were significantly higher in Vertisol than Luvisol. On average, earthworm abundance ranged from 40.00 to 880.00 ind. m^{-2} and earthworm biomass from 5.65 to 92.51 g m^{-2} in Luvisols and Vertisols, respectively. In total five earthworm species were identified in Vertisols, however the Luvisol sites harbor only one species.

3.6. New scientific results

1. The patterns of SMR and earthworm (abundance, biomass, and species richness) of selected major soil groups of Hungary by grouping soils into mollic and non-mollic categories using diagnostic approach were generated. Diagnostic features associated with mollic horizon, i.e., higher SOM, basic cations, MC, and available nutrients positively influence the SMR. On the other hand, a specific association of these diagnostic features and earthworm communities could not be statistically established, suggesting land use type probably had more influence on earthworm communities than soil type in the study area.

2. For the first time, the bacterial community structure and trend of SMR of dominant agricultural soil types of Ethiopia (Nitisols, Vertisols, Luvisols, and Cambisols) were determined.
   i. The highest bacterial genera and SMR was found in Nitisol where high content of P_2O_5, SOC, and exchangeable Mg^2 recorded.
   ii. Genus *Gailla* (from phylum *Actinobacteria)*, *Thermomicrobium roseum* and unclassified *Bacterium Ellin-6519* (from phylum *Chloroflexi),* and order *Rhizobiales* (from phylum *Proteobacteria)* were the most abundant bacterial groups in the studied soils.
   iii. From the identified PGPB, *Streptomyces*, *Sphingomonas*, *Ralstonia* (genus), *Rhizobiaceae*, *Frankiaceae*, *Devosiaceae* (family) were relatively abundant.
   iv. The abundance of essential PGPB was low (<1%), indicating the importance of adopting proper soil managements that encourage the elevation of SOM and P availability to enhance the important agrobacterial community for better crop yield.
3. Bacterial community structure and trend of SMR were compared among similar RSGs of Hungary and Ethiopia. Soils originated from

some geographical location with different RSGs showed more similar bacterial composition than soils with the same RSG but belong to a different country. Site specific soil attributes particularly SOM and MC found to be the key soil properties that discriminate soils among the two countries.

4. CONCLUSIONS

The results of soils from Hungary showed that SMR was greatly influenced by soil type. There was differential effect of LUT on soil physicochemical properties and SMR that varied depending on the site and the time of sampling. Among the physicochemical variables, NO_3^--N, total N, K_2O, SOM, were significantly influenced by LUT. Soil depth had significant influence on NH_4^+-N, NO_3^--N, and total N), CEC, and Mg^{2+}. Soil microbial respiration greatly differed among the two soil depths (0–10 and 10–25cm) with a higher rate of SMR in surface soils compared to subsurface soils.

The earthworm biomass and abundance varied significantly across soil and LUTs, however, explicit correlations with any of soil property measured was not observed. Generally, compared to arable and forest sites, grassland sites were more favorable for earthworm communities.

The results of soils from Ethiopia revealed that Nitisols had higher microbial activity and bacterial richness compared to Cambisols, Luvisols, and Vertisols. The high amount of P_2O_5, soil organic carbon (SOC), and exchangeable Mg^{2+} may attribute to high microbial communities in Nitisol soils. The bacterial community was dominated by *Actinobacteria*, *Chloroflexi*, and *Proteobacteria* phyla in all four soils but in different abundance. The abundance of well-known plant growth promoting bacteria (PGPB) like *Bacillus*, *Pseudomonas* was low (<1%).

Based on comparison result, the rate of SMR was higher in both Luvisol and Vertisol of Hungary compared to Luvisol and Vertisol of Ethiopia. The *Actinobacterial* and *Chloroflexi* phyla highly dominated the Ethiopia soils while *Proteobacteria* was prevalent in Hungarian soils.

6. RELATED PUBLICATIONS

Weldmichael, T.G., Michéli, E., Fodor, H., Simon, B. (2020): The Influence of Depth on Soil Chemical Properties and Microbial Respiration in the Upper Soil Horizons. *Eurasian Soil Science*, 53 (6), pp.780–7866. doi:10.1134/S1064229320060137 (Q2)

Weldmichael, T.G., Márton, D., Simon, B., Michéli, E., Reda, G.T., Adiyah, F., Cserháti, M. (2021): Bacterial community characterization and microbial respiration of selected arable soils of Ethiopia. *Eurasian Soil Science* (accepted with minor revision) (Q2)

Weldmichael, T.G., Szegi, T., Denish, L., Gangwar, R. K., Michéli, E., Simon, B. (2020): The patterns of soil microbial respiration and earthworm communities as influenced by soil and land use types in selected soils of Hungary. *Soil Science Annual*, 71(2), 43–52. doi:10.37501/soilsa/122408 (Q2)

Weldmichael, T.G., Michéli, E., Simon, B. (2021): The response of soil physicochemical properties and soil microbial respiration to different land use types: A case of areas in Northern Hungary region. *Agrochemistry and Soil Science*, 1–16. doi:10.1556/0088.2021.00079 (Q4)

Weldmichael, T. G., Simon, B., Micheli, E. (2018): The challenge and prospects of water resource management in Ethiopia. International Conference on Water Science- "Adaptable Water Management: Opportunities and Threats", March 22, Szarvas, Hungary. ISBN 978-963-269-735-2

Tsedekech Gebremeskel, Lubangakene Denish, Barbara Simon, Erika Micheli (2018): The role of Earthworms in Soil Carbon Dynamics. A review. 3rd International Young Researcher Scientific Conference- "Sustainable Regional Development Challenges of Space & Society in the 21st Century",

April 26, Szent István University, Gödöllő, Hungary. ISBN 978-963-269-730-7

Lubangakene Denish, Ahsan Raza, **Tsedekech Gebremeskel**, Tamás Szegi, Barbara Simon (2018): Assessing the impact of land use system and management on soil properties. 3rd International Young Researcher Scientific Conference- "Sustainable Regional Development Challenges of Space & Society in the 21st Century", April 26, Szent István University, Gödöllő, Hungary. ISBN 978-963-269-730-7

Weldmichael, T.G., Denish, L., Simon, B., Szegi, T., Michéli, E. (2019): Soil Moisture Content is Governed by a Combination of Soil Texture and Soil Organic Matter in Selected Soils of Hungary, 21st Century Water Management in the Intersection of Sciences, March 22, Szarvas, p 366–372Hungary. ISBN 978-963-269-808-3

Gangwar, R.K., Makádi, M., Michéli, E., **Weldmichael, T.G**., Szegi, T. (2017): Impact of soil types and management practices on soil microbiological properties - a case study in salt affected area of Hungary. EGU Scientific Conference, April 24, 2017, Vienna, Austria. (Abstract)

Weldmichael, T.G., Simon, B., Michéli, E. (2019): Major soil groups of Ethiopia: Classification, properties, and management. International Seminar on Environmental Issues & Challenges in the 21st Century (EICC-2019), January 22, 2019, New Delhi, India. (Abstract)

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