MAGYAR AGRÁR- ÉS
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RAVI KUMAR GANGWAR

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MICROBIOLOGICAL AND CHEMICAL CHARACTERIZATION OF HUNGARIAN AND INDIAN SALT-AFFECTED SOILS

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RAVI KUMAR GANGWAR

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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):	Dr. Tamás András Szegi, Institute of Environmental Sciences, Department of Soil Science.

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

1. INTRODUCTION AND OBJECTIVES

Salinization and sodification has become severe threat in both places i.e., Hungary and India, it affects physico-chemical, biological, and biochemical properties of soil (Gill et al. 2009; Rietz and Haynes 2003; Singh et al. 2013b) causing major problems for crop productivity to a significant extent (Gill et al. 2009). In Hungary, approximately 10% of the total geographical area is covered with salt-affected soils (SAS) (Szabolcs & Várallyay 1978). The country exhibits the distinctive traits of continental salinization, sodification and alkalinization as a result of its geological and hydrological conditions, rather than marine influences (Tóth, 2010). While in India, the extent of salt affected soil are estimated to be 6.73 Mha which results in economic losses of $US ~ 3.0 billion annually. Future forecasts indicate that the area of SASs will expand to ~16 Mha by 2050 as a result of improper irrigation practises and climate change (Kumar et al., 2022).

There are many problems associated with soil salinization and sodification like land degradation, desertification and land abandonment which could be the cause of excessive salt accumulation, however, just limited quantities of salt are necessary for both pedogenic and biological processes. The negative impacts of soil salinity and alkalinity are only noticeable when they reach a moderate to extreme level (Wijnja and Bruggenwert, 1994). Thus, the knowledge of the nature of salt affected soils and their distribution, and the understanding of the processes that lead to the soil salinization should not be underestimated. Also, it is important to understand the role of land use and soil types on soil microbes in salt-affected soils, as soil microbial activity plays a key factor in the biodegradation of organic matter (Qualls and Haines 1992), carbon sequestration (Buckeridge et al. 2020), nutrient cycling, energy transformation (Coleman et al. 1983), formation of soil structure (Elliott 1986; García-Orenes et al. 2010) and plant protection (Rahman et al. 2018).

Hence, the purpose of this study was to understand the role of different land use types (arable land, pasture land and meadow) on some major 'salt-affected' soil groups of Hungary by soil physical, chemical and microbiological properties including microbial community structure of salt-

affected soils (Solonetz and Solonchak) and slightly salt-affected soils (Gleysol and Chernozem), and India by soil physical, chemical and microbiological properties of salt affected soils (Solonetz) on arable land, pasture land and bare land in order to verify whether there is a significant difference in aforesaid soil properties in relation to different land use types. The objectives of the study are:

1. To investigate and compare the microbiological and chemical properties of salt-affected soils under different land uses, and thereby to provide relevant information for other salt-affected areas with a similar soil type.
2. To compare the effects of different land uses (arable, pasture, meadow and bare) of different types of salt-affected soils developed under different geographical and climatic conditions in Hungary and in India.
3. To determine the chemical, physical and microbiological properties of salt-affected soils considering the relationship between the biotic and abiotic properties of cultivated and non-cultivated salt-affected soils.
4. To investigate the impact of different land use system on several indicators of soil microbial activity in different soil types and to determine the main driving factors of microbial properties of salt-affected soils.
5. To understand the effects of land use and soil types on microbial activity and community structure of selected Hungarian sites.
6. To investigate the possible effects of different land use types and soil properties on the bacterial community structure of salt-affected soils of Hungary.

2. MATERIALS AND METHODS

2.1 Study area

The study was carried out at two different locations situated in other continents and climate viz. Hungary and India. Sampling site characterization and their abbreviation used are summarized in Table 1.

Table 1: Soil reference groups and land use types of the studied sites

Location	Sampling Site	Soil reference group	Land use type	Abbreviation used
Hungary	Nádudvar (N)	Solonetz (Sn)	Arable (A) land	NSnA
			Meadow (M) land	NSnM
		Chernozem (Ch)	Arable (A) land	NChA
	Apaj (A)	Solonchaks (Sc)	Pasture (P) land	AScP
	Szappanszék (S)	Gleysol (Gl)	Pasture (P) land	SGlP
India	Kittauna (K)	Solonetz (Sn)	Arable (A) land	KSnA
			Pasture (P) land	KSnP
			Bare (B) land	KSnB

a. **Hungary**: Soil samples were collected from Nádudvar (Hajdú-Bihar County) (NSnA; NSnM; NChA), Apaj (Pest County) (AScP) and Szappanszék (Bács-Kiskun county) (SGlP) in Hungary (Figure 1). The climate of the sampling sites was typical European continental/Pannonian with warm, dry summers with annual temperature ranges from 9.8 to 10.5 °C and annual precipitation ranges from 510 to 550 mm.

   The Hungarian sites encompassed three land use types viz. arable land, pasture land and meadow land. The cultivated arable sites (NSnA, NChA) were ploughed to a depth of 30 cm and 400 kg ha^{-1} NPK (18:7:7) fertilizer was applied to the maize crop. The non-ploughed pasture site (NSnM) has not been cultivated for more than 30 years, while the Apaj site (AScP) was grazed by sheep and this site received grazed animal droppings and both (NSnM and AScP) sites had almost continuous grassy vegetation. While

Szappanszék (SGlP) is a drying saline lake occasionally flooded representatively during the early spring times, with continuous grassy vegetation and extensive Hungarian gray ox grazing. The site has been protected since 1975 and belongs to the Kiskunság National Park.

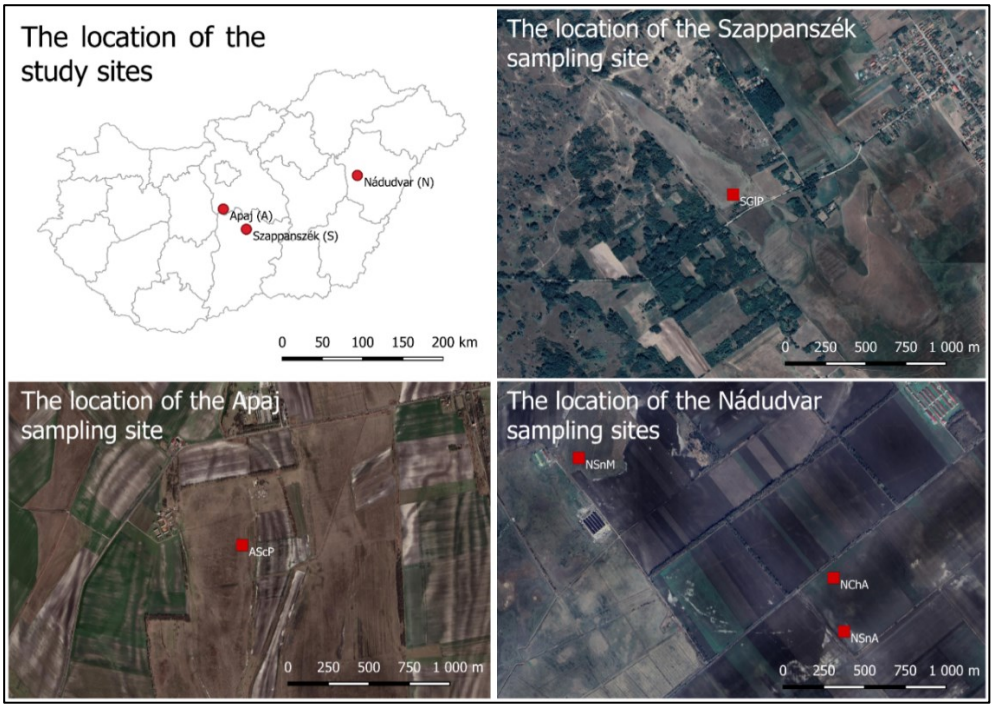

Figure 1. Location of the sampling sites in Hungary

b. **India:** In India, the studied area was located in Kittauna (K) village present in Aonla, Bareilly district of Uttar Pradesh. Three land use types viz. arable (KSnA) land, bare (KSnB) land and pasture (KSnP) land was used for the study (Figure 2). The climate of Indian site is sub-tropical with mean annual temperature of 25.1 °C and total annual rainfall is 1037 mm, mostly occurring during July–September. The arable land was ploughed to the depth of 30 cm and black gram (*Vigna mungo*) was produced, whereas bare land (which was an arable land 30 years ago) had less than 10%, and pasture land had 50% grassy vegetation cover. Based on inherent practices arable land was fertilized by Urea whereas pasture received the grazed animal droppings.

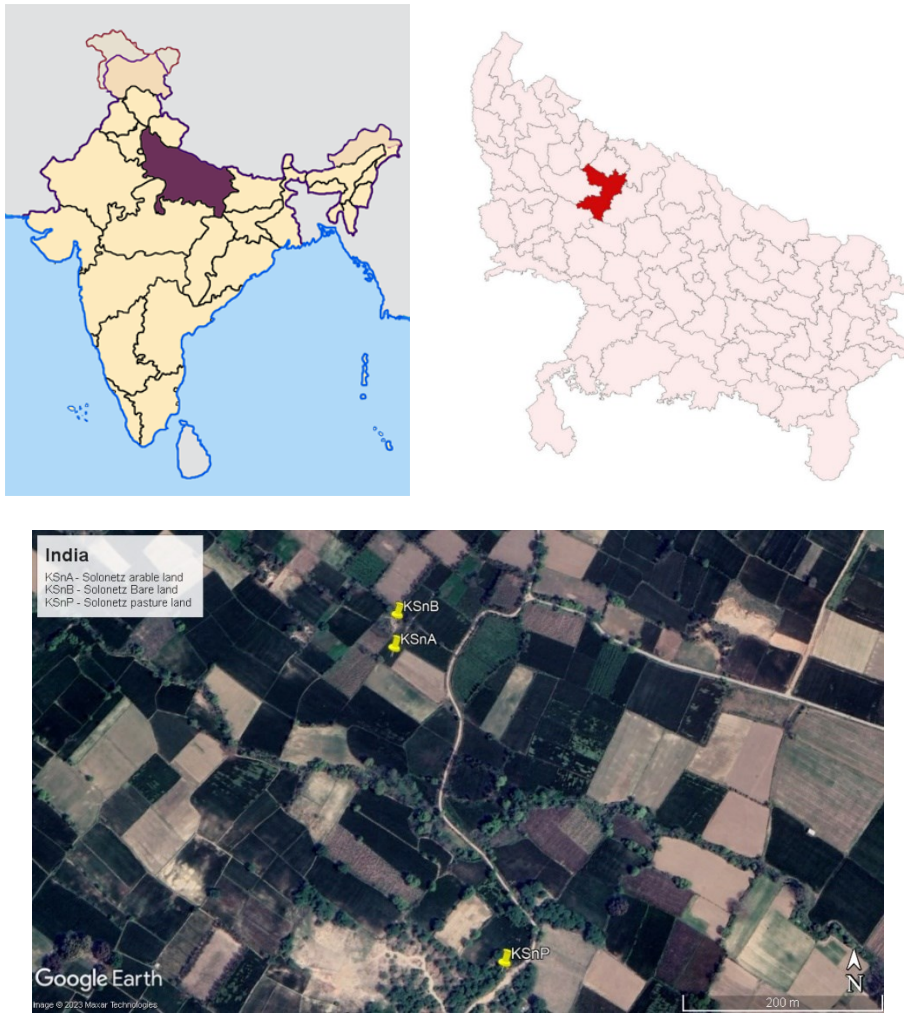

Figure 2. Google Earth maps showing soil sampling locations of India.

2.2 Soil sampling

In Hungary, soil samples were collected in the month of May 2016 and September 2016 followed by another sampling in the month of May 2017. The soil samples were collected from the upper surface layer (0-15 cm depth). For collection of soil samples, eight plots of 100 m^2 were selected from each site namely AScP, NSnA, NSnM, NChA and SGlP (Figure 3). Ten soil subsamples were randomly collected and combined to make a well-mixed composite sample from each plot. All the vegetation and litter from the soil surface was removed before sampling. Collected soil samples were placed in plastic bags and transported back to the laboratory in a cooling box. Samples

were sieved through a 2 mm sieve to get a well-homogenized sample and stored at -20 °C. Before analysis, soils that were analyzed for microbial biomass carbon (MBC), enzymes activity (dehydrogenase-DHA, phosphatase) and basal soil respiration (BSR) were placed in 4 °C for one night. For chemical analysis the sieved soils were air dried and stored at room temperature (22-24 °C). Also, soil profile samples were collected for soil classification. Samples from different soil horizons were sieved (<2 mm), air dried and stored for chemical and physical analysis. Table 2 represents the sampling strategy for 2016 and 2017, and the measurements carried out for sampling done in both years. For 2017 sampling, samples were collected from two plots of 100 m^2 based on the highest and the lowest observed microbial biomass carbon.

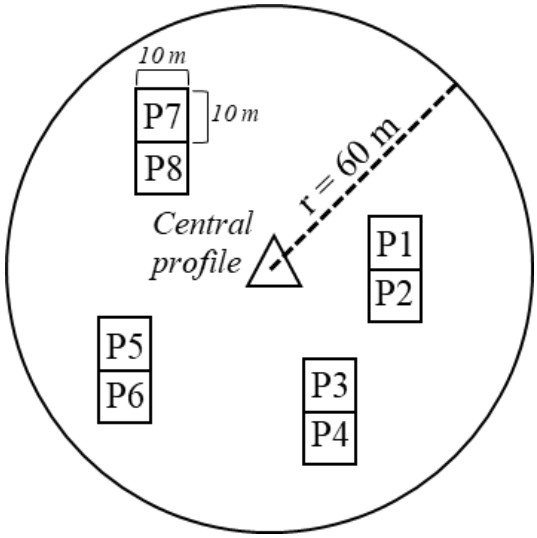

Figure 3. Schematic figure of plots and soil profile within the sampling sites of Hungary (NSnA, NSnM, AScP, NChA and SGlP).

Table 2: Parameters analysed for each sampling year

Year	Sampling site	Samples collected	Parameters analysed	
			Physico-Chemical properties	Microbiological properties
May 2016	AScP, NSnA, NSnM, NChA and SGlP	40 samples (8 samples from each site)	OC, pH, EC P_2O_5, K_2O, Mg^{2+}, Ca^{2+}, Na^{+}, Moisture	BSR, MBC, DHA Phosphatase
September 2016	AScP, NSnA, NSnM, NChA and SGlP	40 samples (8 samples from each site)	OC, pH, EC P_2O_5, K_2O, Mg^{2+}, Ca^{2+}, Na^{+}, Moisture	BSR, MBC, DHA Phosphatase
May 2017	AScP, NSnA, NSnM, NChA and SGlP	10 samples (2 samples from each site)	OC, pH, EC P_2O_5, K_2O, Mg^{2+}, Ca^{2+}, Na^{+}, Moisture	BSR, MBC, DHA Phosphatase PLFA properties
		5 samples (1 sample from each site)	Soil texture	Illumina 16S rRNA gene amplicon sequencing

In India, Soil sampling was performed twice in the year 2016 viz. in the month of March and October, respectively. For collection of soil samples, four plots of size 100 m^2 were selected from bare land (KSnB) and pasture land (KSnP) each and eight plots of same size were selected from arable land (KSnA) (Figure 4). Ten soil subsamples were randomly collected and combined to make a well-mixed composite sample from each plot. All the vegetation and litter from the soil surface was removed before sampling. Collected soil samples were placed in plastic bags and transported back to the laboratory in a cooling box. Samples were sieved through a 2 mm sieve to get a well-homogenized sample and stored at -20 °C. Before analysis, soils that were analyzed for microbial biomass carbon, soil enzymes activity and basal soil respiration were placed in 4 °C for a night. For chemical analysis the sieved soils were air dried and stored at room temperature (22-24 °C).

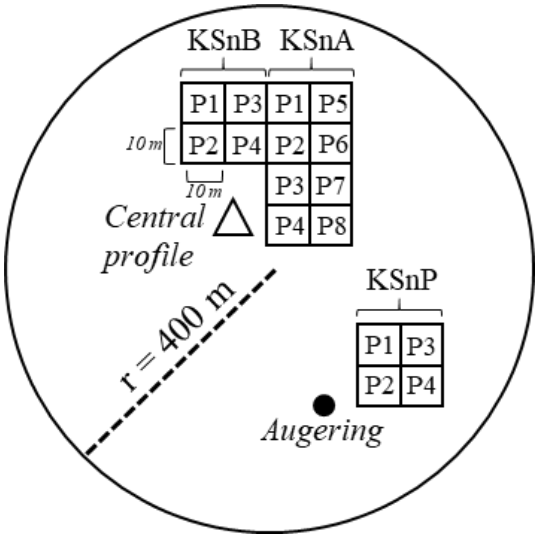

Figure 4. Schematic figure of plots and soil profile within the sampling sites of India (KSnB, KSnA and KSnP); △: location of soil profile; ●: location of augering

2.3 Laboratory analysis

2.3.1 Soil physicochemical analyses

Soil pH was measured in soil-water suspension (1:2.5) while electrical conductivity (EC) was measured using the same soil suspension (Buzás, 1988). Soil organic carbon (%) was determined by the method given by Walkley and Black (1934). Humic material (E4/E6) was determined by the method given by Page et al. (1982). Plant available AL-(ammonium lactate) P_2O_5, AL-K_2O and plant available nutrients (avNa^+, avCa^{2+} and avMg^{2+}) were extracted using Ammonium-lactate/-acetic acid buffer solution (0.1 M; pH=3.7) according to Egnér et al. (1960). Soil moisture content was determined using the gravimetric method and particle size distribution was determined using pipette method (Buzás, 1993).

In case of the soil profiles, samples from different layers were sieved (<2 mm), air dried and stored for chemical and physical analysis. The chemical analysis of organic carbon (OC), electrical conductivity (EC) and pH were determined by above mentioned methods whereas exchangeable basic cations (S value) were determined based on the modified Mehlich method (Mehlich, 1953)

where, exchangeable sodium percentage (ESP %) was calculated as the following: exchangeable sodium ($exNa^+$) / (sum of $exNa^+$, $exCa^{2+}$, $exMg^+$ and exK^+) *100. While pipette method (Buzás, 1993) was used to determine particle size distribution.

For soil classification, profile samples were analysed. The above-mentioned methods were used for the chemical analysis of organic carbon (OC), EC and pH, whereas cation exchange capacity (CEC) and exchangeable basic cations (S value) were determined based on the Mehlich method (Mehlich, 1953). The exchangeable sodium percentage (ESP %) was calculated as exchangeable Na / CEC *100 (USDA 1954). The $CaCO_3$ content was measured with the Scheibler gas-volumetric method (Buzás, 1988), while particle size analysis was conducted using the pipette method (Buzás, 1993).

In each location one central soil profile was described (FAO, 2006) and classified according to WRB 2014 updated 2015 (IUSS Working Group WRB 2014) to characterize the pedological conditions and in India one additional augered profile was open to confirm the presence of the similar soil type for the Indian pasture site (KSnP). Table 1 presents the soil reference groups and land use types of the studied sites.

2.3.2 Soil microbiological analyses

Soil microbial biomass carbon (MBC) was estimated by the chloroform fumigation-extraction method (Brookes et al., 1985; Vance et al., 1987). Briefly, six portions equivalent to 12.5 g fresh soil were taken from each soil sample. Three portions were fumigated in vacuum desiccators for 24 h at 25 ºC with ethanol free $CHCl_3$ containing boiling chips in the center of the desiccator. Paper towels, moistened with deionized water, were also placed in each desiccator to help maintain the water content of soils during fumigation. After the chloroform was removed, the soil was extracted with 25 ml 0.5 M K_2SO_4 by horizontal shaking for 30 minutes on a mechanical shaker and then filtered. At the same time, unfumigated soil samples were placed in the bottles and were treated in the same way and was used as controls. Microbial biomass carbon in filtrates was then determined by potassium dichromate method.

Microbial activity or basal soil respiration (BSR) represents the feedback of microbes against entering of organic substrate. The alkali absorption method

was used to measure BSR. (Carter, 1993; Cheng et al., 2013). Briefly, 50 g fresh soil was adjusted to 60% field capacity and placed in an airtight jar (1 l capacity). Soil moisture content in the jar was adjusted with deionized water. At the same time, a glass conical of 50 ml capacity containing 1.0 M NaOH was installed in the jar to trap respired CO_2. After 10 days, the conical was removed and excess $BaCl_2$ was added in the NaOH solution to precipitate the trapped CO_2 as insoluble $BaCO_3$. The NaOH concentration left in the conical was titrated with 1 M HCl solution at the phenolphthalein end point. For control, a same set of experiment was repeated without soil. The basal soil respiration was expressed in mg CO_2 kg^{-1} hr^{-1}.

The activity of phosphatase enzyme was measured as described by Tabatabai and Bremner (1969). This involves colorimetric estimation of the p-nitrophenol released by phosphatase activity. Briefly, 1.0 g soil was incubated in modified universal buffer solution (pH 11.0) with para-nitrophenyl phosphate substrate at 37 °C. After 1 h, reactions were stopped with 0.5 M NaOH, filtered with Whatman 42 paper and the formation of p-nitrophenol determined colorimetrically using a spectrophotometer at 400 nm.

Dehydrogenase activity (DHA) was determined by the transformation of 2,3,5-triphenyltetrazolium chloride (TTC) to 1,2,5- triphenylformazan (TPF) (Casida et al., 1964). A 5 ml aliquot of TTC-Tris buffer solution was added to 5 g of soil in 50 ml glass flasks. After 24 h incubation at 37 °C, the reaction product was extracted with ethanol. The formation of triphenylformazan (TPF) was determined spectrophotometrically at 485 nm and results were expressed as g TPF g^{-1} dry sample.

2.3.3 Community analysis by Phospholipid fatty acid (PLFA)

Phospholipid fatty acid (PLFA) analysis was done on the samples collected from all selected sites of Hungary in 2017. Two plots from each site were selected for analysis based on the highest and the lowest microbial biomass carbon observed in all investigated sites during 2016. PLFA indicator molecules were determined from soil samples based on a modified method of White et al. (1979). The prepared samples were stored at –20ºC until an analysis was performed using a gas chromatograph-mass spectrometer system (GC 6890N with MS 5975, Agilent, Santa Clara, CA, USA) with a 100 m

Supelco SP-2560 column, in selected ion mode and scan mode as well (50-350 amu). For PLFA identification methyl nonadecanoate was used as an internal standard. The unbranched, saturated PLFAs such as C14:0, C15:0, C16:0 and C18:0 were used as general bacterial markers. Branched, saturated PLFAs iC15:0, aC15:0, iC16:0, iC17:0 and aC17:0 were used to indicate Gram-positive bacteria. Gram-negative bacteria were characterized using monoenoic and cyclopropane with unsaturated C18:1n9c and cyC19:0 PLFAs (Gude et al., 2012). 10MeC16:0 and 10MeC17:0 PLFAs were used to quantify Actinobacteria (Dong et al., 2014) and C16:1n5c for arbuscular mycorrhiza fungi (AMF) (Marshall et al., 2011). Polyunsaturated C18:2n6c, C18:3n6c and c18:3n3 were used as Fungi markers (Nakatani et al., 2012). The total PLFA content was calculated as the sum of the abovementioned PLFAs. Moreover, the ratios of Gram-negative to Gram-positive Bacteria, Fungi to General Bacteria and Actinobacteria to General Bacteria were calculated.

2.3.4 Illumina 16S rRNA gene amplicon sequencing and bioinformatics analysis

Illumina 16S rRNA gene amplicon sequencing was used to precisely assess the bacterial community composition of the chosen soil samples of May 2017. One sample was selected from each site based on the microbiological analyses. For this, community DNA was extracted from the composite soil samples using the NucleoSpin Soil Mini Kit (Macherey-Nagel), according to the instructions of the manufacturer. Subsequently, for paired-end 16S rDNA amplicon sequencing, the variable V3 and V4 regions of the 16S rRNA gene were amplified using forward (5′-TCGT CGGCAGCGTCAGATGTG TATAAGAGACAGCCTA CGGGNGGCWGCAG-3′) and reverse (5′-GTCT CGTGGGCT CGGAGATGTGTATAAGAGAC AGGACTACHVGGGTATCTAATCC-3′) primers with Illumina adapter overhangs (Klindworth et al., 2013). PCR mixtures contained 12.5 ng of DNA, 0.2 µM of each primer and 12.5 µL of 2X KAPA HiFi HotStart Ready Mix (KAPA Biosystems, London, UK) supplemented with MQ water up to 25 µL final volume. The temperature profile was the following: initial denaturation for 5 min at 95°C, 25 cycles of amplification (30 s at 95°C, 30 s at 55°C, 30 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 (Life Technologies, Carlsbad, USA). Amplicons were analysed by agarose gel electrophoresis. Paired-end fragment reads were generated on an Illumina MiSeq sequencer using MiSeq Reagent Kit v3 (600-cycle). 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 the threshold. Trimmed sequences were processed using mothur v 1.41.1. as recommended by the MiSeq SOP page (https://www.mothur.org/wiki/MiSeq_SOP). Paired-end sequence (contig) numbers ranged between 45323 and 49853. The sequence assortment based on the alignment with the SILVA 132 SSURef NR99 database. Chimera detection was performed with the mothur's uchime command. The 'split.abund' command was used to remove singleton reads. The standard 97% similarity threshold was used to determine operational taxonomic units (OTUs) as it was suggested for prokaryotic species delineation (Tindall et al., 2010). Rarefaction curves were also generated and showed high sequencing coverage in all samples Raw sequence reads were deposited in NCBI SRA under BioProject ID PRJNA760983. The 20 most abundant OTUs were identified using the EzBioCloud 16S rDNA database.

2.4 Statistical analysis

For comparative analysis of Hungarian and Indian samples, in case of soil chemical and physical properties, the analyses of variance (ANOVA) of the data from different sites were computed using SPSS statistics vs 23.0. The mean of parameters of different sites were separated using Tukey HSD post hoc test at $p < 0.05$ level. The chemical and physical properties of all composite samples were applied to calculate the Principal Component Analyses (PCA) using PAST 4.05 software. One-way ANOSIM was used to determine the differences among the sites. Clustering the sites based on their microbiological properties Bray-Curtis analysis was carried out (PAST vs. 4.05). A canonical correspondence analysis (CCA) was performed to predict the relationships between the microbiological properties and the environmental factors of the studied sites.

Similarly, for selected samples from Hungarian sites, ANOVA was computed using same statistical package (SPSS statistics vs 23.0). The Tukey HSD post hoc test was used to separate the means of parameters from different sites at a significance level of $p < 0.05$. The chemical and physical properties of all composite samples were utilized to perform Principal Component Analysis (PCA) using PAST 4.05 software. The sites were clustered using the Bray-Curtis analysis, based on their microbiological properties. A CCA was then conducted to determine the connections between the microbial properties and environmental factors at the sites (using PAST vs. 4.05). Correlation between the TOP20 OTUs of soil samples (revealed by Illumina amplicon sequencing), environmental variables, and sampling areas was calculated with canonical correspondence analysis (CCA) using PAST 4.05 software.

3. RESULTS AND DISCUSSION

3.1 Effect of land use on soil properties of salt affected soils of India and Hungary (year 2016)

3.1.1 Effect of land use on soil chemical and physical properties

Results of soil chemical and physical properties indicated that OC, AL-P_2O_5, AL-K_2O and moisture content were higher at Hungarian sites, while, pH, avMg^{2+}, avCa^{2+} and avNa^{+} were higher in case of Indian sites. Based on the investigated chemical parameters and soil moisture content, the six sites are statistically different from each other.

The result of the principal component analysis (PCA) of Hungarian and Indian soils (Figure 5) showed that the component 1 and component 2 retained together accounted for more than 91% of the total variance. The first component was determined positively by AL-P_2O_5 and negatively by avMg^{2+} which explains more than 82% of the variance and clearly separates the Hungarian and Indian locations. While the second component explains more than 9% of the variance and determined positively by AL-P_2O_5, avCa^{2+} and negatively by avNa^{+}.

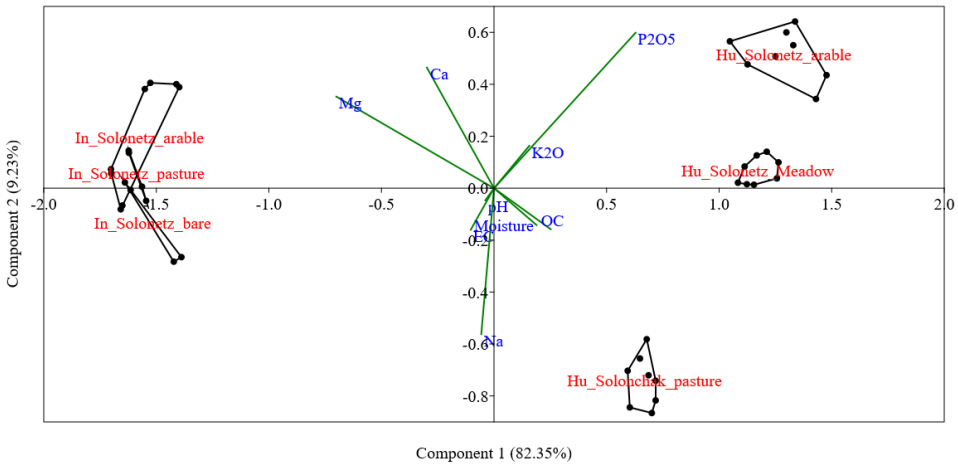

Figure 5. Results of the Principal Component Analysis (PCA) based on the chemical properties and moisture content of Hungary and India (from two sampling seasons of year 2016).

3.1.2 Effect of land use on soil microbiological properties

The measured soil microbiological parameters are regularly used indicators for investigating soil health and fertility (Alhameid et al., 2019), revealing high differences between Hungarian and Indian salt-affected soils. Soil microbiological parameters showed lower values in case of Indian sites, except for the BSR. The higher values of microbiological parameters (MBC, DHA and Phosphatase enzyme activity) in Hungarian soils could be due to higher organic matter, moisture and higher macronutrient content and preferable pH i.e., from slightly acidic to slightly alkaline (Figure 6).

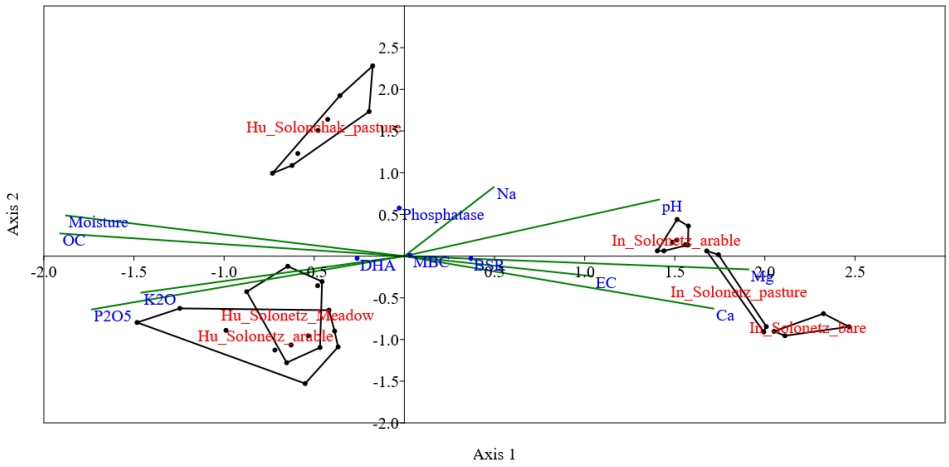

Figure 6. Canonical Correspondence Analyzes (CCA) of biological and environmental factors of the sampling sites of Hungary and India (from two sampling seasons of year 2016 samples).

Canonical Correspondence Analyzes (CCA) was used to determine the main environmental parameters affecting microbiological properties (Figure 6). Our results showed that more than 86% of variation in microbiological properties are caused by abiotic properties. At Hungarian sites (NSnA and NSnM), soil OC, moisture, K_2O and P_2O_5 were the main positive factors effecting DHA while in case of Indian Solonetz soil sites (KSnA and KSnP), variations in BSR were positively influenced by pH, EC, avMg and avCa (Axis 1). Whereas Hungarian Solonchak pasture (AScP) was characterized by phosphatase enzyme activity (Axis 2).

The cluster analysis (Bray-Curtis) (Figure 7) of soil microbiological properties showed that Hungarian and Indian sites are separated from each other. In Hungary it seems that the land use had more pronounced effect on clustering, then the inherited soil chemical properties, and/or soil reference groups (Solonetz / Solonchaks) as the pasture sites (AScP, NSnM) are very similar to each other, while the Hungarian arable sites (NSnA) formed a separate cluster.

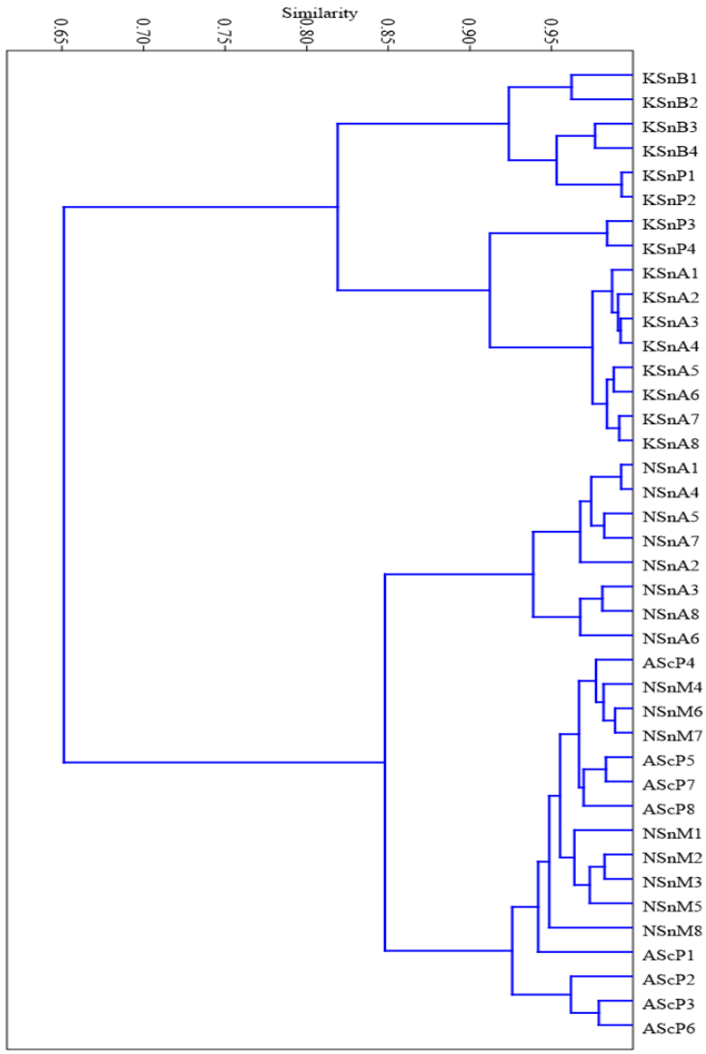

Figure 7. Cluster analyzis (Bray-Curtis) of the sampling sites of Hungary and India based on the microbiological properties (from two sampling seasons of year 2016 samples).

3.2 Effects of land uses and soil types on soil properties of Hungarian soils (year 2017).

3.2.1 Effects of land use and soil types on soil chemical, physical and microbiological properties (including PLFA)

The interaction between land use and soil chemical properties were investigated by principal component analysis (PCA, Figure 8). The component 1 and component 2 explained 71.69% and 21.50% of the total variance, respectively. The effect of land use was reflected on component 1 with positive values for arable land and meadow land in the centre, and negative for pasture land. The first component was determined positively by P_2O_5 and Ca while the second component was positively reflected by EC and Na. Specifically, the arable lands (NSnA and NChA) had higher amounts of plant available P_2O_5 and Ca, while the pasture land (AScP) could be characterized with high EC and Na content. The different soil types and land uses could be separated clearly.

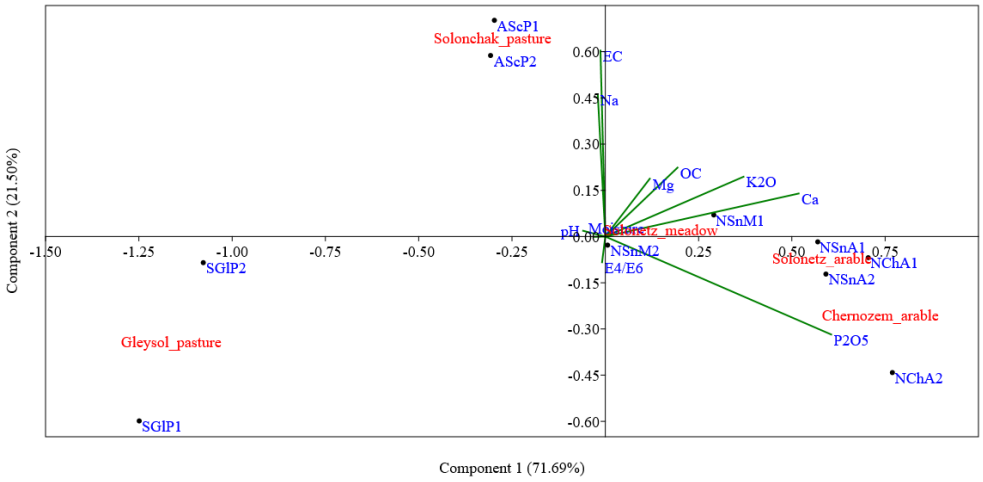

Figure 8. Results of the principal component analysis based on the chemical properties and moisture content of investigated Hungarian soils grouped by soil types and land uses from year 2017 samples.

3.2.2 Effects of land use and soil types on PLFA composition

Analysing the microbiological properties of studied sites revealed some similarities and dissimilarities. For deeper analysis of this question, Cluster

analysis using Bray-Curtis distance measure was carried out with all the measured microbiological properties, which revealed that the sampling sites were separated into two main clusters based on the microbiological properties: slightly-salt-affected (NChA and SGlP) and salt-affected (NSnA, NSnM and AScP) (Figure 9). The Solonetz arable (NSnA), Solonchak pasture (AScP) and Chernozem arable (NChA) sites formed different clusters. NSnM2 site was separated from NSnM1, which is closer to Apaj pasture sites. Chernozem arable (NChA) was also grouped with Gleysol pasture presumably due to the lower moisture and Na^+ content.

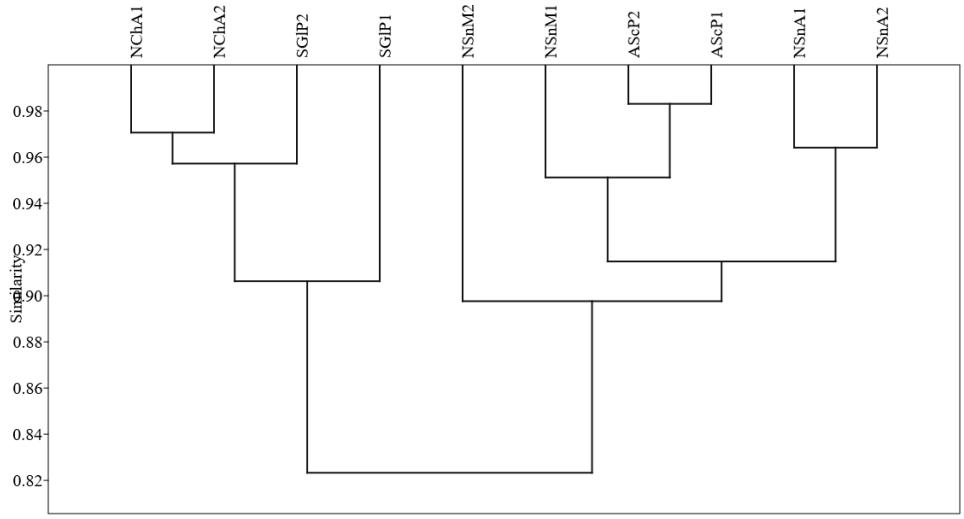

Figure 9. Cluster analysis (Bray-Curtis) of the samples based on the investigated soil biological properties of Hungarian soils from year 2017 samples.

CCA was used to determine the main environmental parameters affecting microbiological properties including PLFA (Figure 10), the first two axes described 47.63 and 30.95% of variance. On Axis 1 the moisture content was the main factor affecting positively soil respiration, microbial biomass carbon, DHA and phosphatase activity while general bacteria, Gram-positive bacteria, Actinobacteria, AMF, Gram-negative bacteria and Fungi were influenced negatively. Whereas on Axis 2 OC, EC, Mg and Na were the main environmental factors affecting positively DHA, phosphatase activity, Actinobacteria, AMF and Fungi while soil respiration, MBC and Total PLFA were negatively influenced. Sampling sites with different soil types and land

use practices distributed near the origin but both arable sites (NSnA and NChA) separated along the first axes together with SGlP2 site while AScP sites separated along the Axes 2 together with SGlP1 site. Loadings of NSnM sites were $P<0.05$.

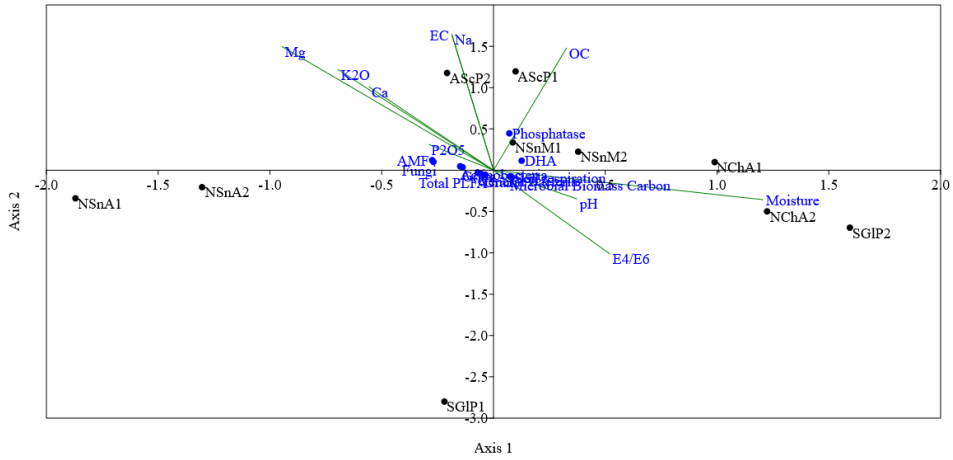

Figure 10. Canonical Correspondence Analysis of the sites in Hungary from year 2017 samples.

3.3 Effects of different land usage on the bacterial community composition

The bacterial communities of the soil samples were dominated by members of phyla *Pseudomonadota* (23-37%), followed by *Acidobacteriota* (17-25%) and *Actinobacteriota* (7-20%). Results of the bacterial community analysis have shown that besides land use and land cover, soil texture had a crucial effect on the presence or lack of some bacterial classes in the communities. The best example of this observation is the different occurrence of classes *Blastocatellia* and *Acidobacteria* in the investigated soils. Both classes belong to the phylum *Acidobacteriota*, and it was observed that members of the class *Acidobacteria* were exclusively abundant in soil NSnM and were marginal in the other samples. Contrarily, members of the class *Blastocatellia* were marginal in soil NSnM but were abundant in the other ones. Our results show that higher sand content may be the main factor, which causes the high abundance of *Blastocatellia* in the *Acidobacteriota* community, rather than the extent of plant cover. The sand content of soil SGIP was extremely high

compared to other soils investigated in this study, and most probably this characteristic caused the high abundance of *Blastocatellia* bacteria here.

Similar to *Blastocatellia*, *Vicinamibacteria* were marginal in soil NSnM, but abundant in the other soil samples. Members of the class *Vicinamibacteria* showed the highest abundance in soils of arable lands (soils NChA and NSnA), and lower abundances in soils SGlP and AScP. As it was mentioned above, members of the class *Acidobacteriia* were exclusively abundant in soil NSnM, which was almost undisturbed meadow soil with high silt and clay content.

Members of *Actinobacteriota* are widely distributed in the soil with high sensitivity to acid and low pH and show maximum growth around neutrality but grow best at a pH between 6 and 9. Class *Actinobacteria* and *Thermoleophilia* were highly abundant in both NSnM and AScP and were marginal in other samples. The relative abundance of both classes was higher in Solonetz meadow comparative to Solonchak pasture. However, the higher abundance of Actinobacteriota at Apaj site was characterized by the sandy soil texture and high sodium content.

Higher relative abundance of *Chloroflexota* was found in NSnM and SGlP. OTUs belonging to class *Ktedonobacteria* of this phylum reached a relative abundance of over 9% in soil NSnM (Table 3), The dominant ktedonobacterial OTU (4.8%) of soil NSnM was most closely related to *Dictyobacter aurantiacus*, although at considerably low level of 16S rRNA gene similarity (86.4%).

The higher abundance of *Gemmatimonadota* was found in soils NChA and NSnA (10.3% and 13.6%, respectively). Similar to *Gemmatimonadota*, members of the class *Phycisphaerae* (phylum *Planctomycetes*) showed the highest relative abundances in soils NSnA and NChA. Members of the class *Verrucomicrobiae* were most abundant in soil NSnM (12%) and the least abundant in soil SGlP (1%). With low soil moisture at SGlP and due to high sand content, bacteria belonging to the phylum *Bacillota* were detected in notable amount in sample SGlP (9%).

Table 3: The TOP20 operational taxonomic units (OTUs) detected in the investigated soils. Taxonomical identification was based on the EzBioCloud 16S rRNA gene database, taking into account valid names only. ND, not detected.

No. OTU	Abundance (%)					Taxonomy	Similarity (%)
	NSnM	AScP	NChA	SGlP	NSnA		
1	2.8	2.3	6.2	1.4	18.7	*Sphingomonas parvus/limnosediminicola*	98.0
2	ND	3.1	3.1	7.5	3.5	*Brevitalea aridisoli/deliciosa*	93.7
3	ND	4.6	2.6	1.9	1.4	*Brevitalea aridisoli*	94.2
4	0.3	1.1	3.2	2.2	0.6	*Sphingomonas aquatilis/melonis/humi*	98.9
5	1.0	0.7	0.8	3.6	1.0	*Bacillus nealsonii/oryzisoli/circulans*	98.9
6	ND	3.3	1.0	0.9	0.5	*Collimonas arenae/Glaciimonas singularis*	92.3
7	ND	3.3	1.7	ND	<0.1	*Azoarcus olearius*	91.0
8	4.8	ND	ND	ND	ND	*Dictyobacter aurantiacus*	86.4
9	4.6	ND	ND	ND	ND	*Acidobacterium ailaaui*	91.6
10	ND	1.1	0.8	2.5	0.3	*Halochromatium roseum*	89.0
11	3.0	0.3	0.2	0.6	0.4	*Bradyrhizobium macuxiense*	99.6
12	ND	0.6	1.5	<0.1	1.7	*Sphingomonas daechungensis*	99.5
13	4.0	ND	ND	ND	ND	*Actinoallomurus purpureus/spadix/vinaceus*	95.1
14	3.9	<0.1	ND	<0.1	<0.1	*Candidatus Solibacter sp.*	98.6
15	3.2	ND	<0.1	ND	0.5	*Roseimicrobium gellanilyticum*	88.8
16	<0.1	1.1	0.7	1.2	0.7	*Stenotrophobacter terrae*	97.3
17	ND	0.5	2.2	0.5	0.5	*Vicinamibacter silvestris*	93.1
18	ND	3.1	<0.1	ND	ND	*Aquihabitans daechungensis*	91.6
19	ND	ND	<0.1	ND	3.2	*Nitrosospira lacus*	90.3
20	ND	0.4	2.3	0.6	0.2	*Methyloversatilis thermotolerans*	90.3

To reveal relationships between the soil bacterial communities, an OTU-based UPGMA dendrogram was created by applying the Bray-Curtis similarity index. On the dendrogram it was clearly observable that bacterial community composition of soil NSnM distinctly differed from that of the other samples, which formed two subgroups according to their land use type. Consequently, one subgroup contained the pasture soils, and another one contained the arable soils (Figure 11). To better understand this grouping of the bacterial communities, Venn-diagrams were generated revealing the distribution of

OTUs among the samples (Figure 12). The highest ratio of shared OTUs (20%) was observed between the arable soil samples NSnA and NChA, followed by the two pasture soils SGlP and AScP (19.2%). The lowest ratio of shared OTUs (6%) could be observed between the meadow soil NSnM and the pasture soil AScP.

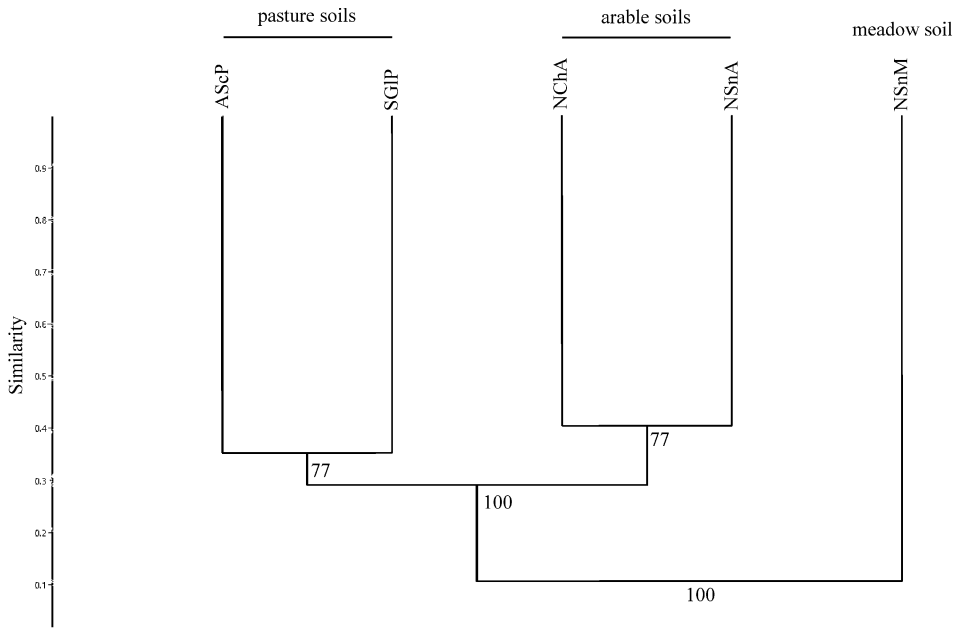

Figure 11. OTU based UPGMA dendrogram of the soil bacterial communities. To generate the dendrogram the Bray-Curtis similarity index was used.

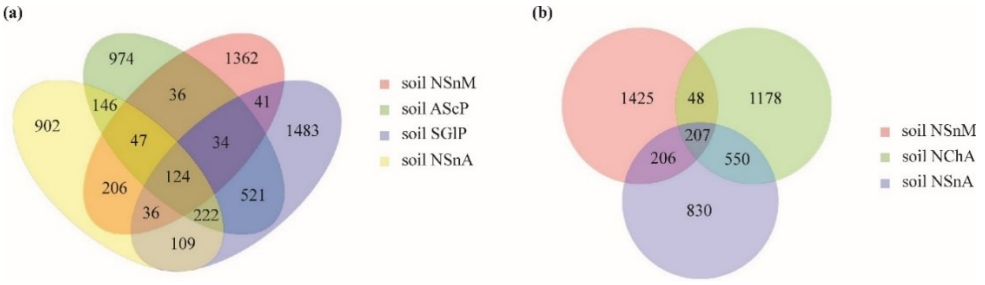

Figure 12. Venn-diagrams showing the unique and shared OTUs among (a) the salt- affected soils, and (b) soils of the ”Nádudvar” site.

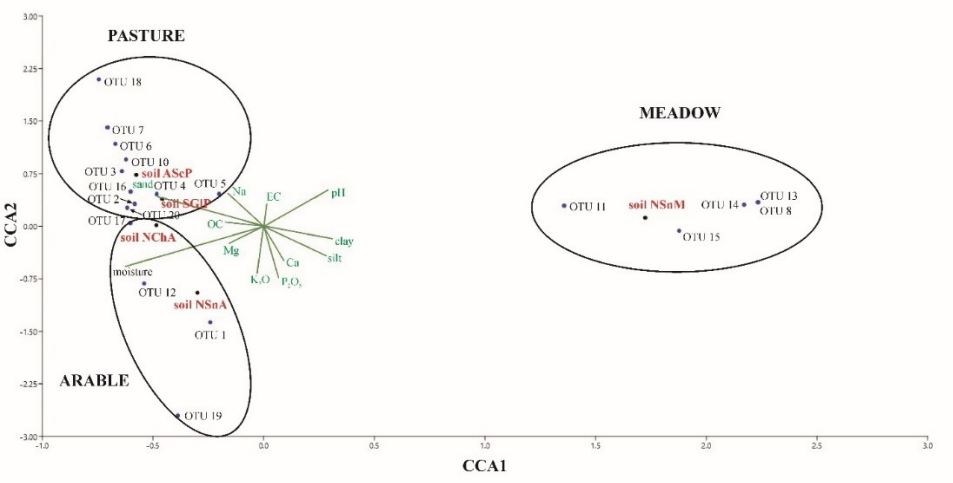

Figure 13. Canonical Correlation Analysis (CCA) between the 20 most abundant microbial OTUs of soil samples, environmental factors and sampling areas.

Further, the canonical correspondence analysis (CCA) based on the soil abiotic parameters and the abundance values of the TOP20 OTUs (see OTU list in Table 3) showed a distinct separation of soil NSnM from the others (Figure 13). Thus, the outlying nature of soil sample NSnM, which was taken from a meadow, was evident again. The sharp separation of this soil sample was caused by the high abundance of OTUs which could be identified as a OTU 8, OTU 14, OTU 13, OTU 14 and OTU 11. Nevertheless, none of the investigated environmental parameters explained the outlying nature of soil sample NSnM. The other four soil samples were grouped closer to each other. Still, the arable soil NSnA and NChA had a slightly separate position on the CCA plot. This separation was caused mainly by the high abundance of *Sphingomonas*-related OTUs (OTU1 and OTU12) and a positive correlation with the soil moisture content was also observable. In case of pasture soil, SGlP OTU 2, OTU 5 and OTU 10 reached their maximum abundance and showed a significant positive correlation ($p<0.05$) with the high sand content of this soil. In soil AScP, OTU 18, which was identified as an *Iaimiaceae*-related actinobacterium and showed a positive correlation with the high Na^+ concentration.

4. CONCLUSION

The sampling sites of the investigated two countries (Hungary and India) are significantly different from each other in their chemical and microbiological characters, despite all the studied sites were characterized as salt-affected soils. The Hungarian sites have preferable soil chemical properties which result in more favorable microbiological parameters comparing with the Indian sites.

In Hungary, the three locations with two different land use practices belonging to two different soil groups can be separated from each other, while Indian sites with different land use practices are slightly overlapping with each other based on the chemical properties. In Hungary, the land use types, the pasture lands and the arable land are clearly separated from each other based on the microbiological properties. Although the pasture sites were described by two different soil reference groups, both are salt-affected ones (Solonetz and Solonchak). In case of the microbiological properties, the land use has a stronger driving force than the original/inherited soil properties. Concerning to the investigated microbiological properties of the three different Indian land use practices, they are forming only two main clusters, arable and bare, as the pasture land samples are grouped to arable and bare clusters as well. Presumably, overusing of salt affected arable lands over a long period of time resulted in land use change to pasture and bare land which leads to the abandonment of those lands to revive naturally. Under the same management practices, the arable sites, which were characterized by the most favorable properties among the Indian sites can face with the similar degradation process in the future and can be abandoned.

Further analysis of Hungarian soils was studied with different soil types (Solonetz, Solonchak, Glesols and Chernozems) under different land use practices (Arable, Pasture and Meadow) to understand how the land use practices and soil types affected the soil physical and chemical differences and also to find the main driving factors of soil microbial properties.

Principal component analysis of the chemical properties of the soil proved that the sites could be grouped according to the land use and soil type. Cultivating

Chernozem soils as arable land could decrease the size of its microbial community to a third of the microbial community size of the salt-affected Solonetz meadow and Solonchak pasture lands. However, the measured soil chemical parameters were different among sampling sites and P_2O_5 played a key role in site differentiation, the microbial properties were mainly determined by soil moisture content, according to the canonical correspondence analysis results.

Based on all of the microbiological properties studied including phospholipid fatty acid, the salt affected soils formed a well separated cluster as opposed to the other soil classification units which were slightly-salt affected soils. Soil types may be the driving factor as salt-affected soils and slightly salt-affected soils are far away from each other in terms of taxonomic distances, for soil groups with short taxonomic distances, land use had more pronounced effects on soil microbiological properties. Continuous plant coverage and the decreased mechanical disturbance of the soil may preserve and/or improve soil function which was proven by our microbial and chemical results. Preserving and enhancing the organic matter content of our soils will improve their microbiological properties.

Furthermore, it was observed that at arable lands, the cultivated plant (maize at the Nádudvar site) and the usage of fertilizers caused low bacterial diversity and the high abundance of some characteristic maize rhizosphere-associated bacteria (e.g. *Sphingomonas* spp.) and ammonia oxidizers (e.g. *Nitrosopsira*-related bacteria), respectively. At those sites where the salt-affected soil was not disturbed (pasture and meadow soils), soil texture together with the ratio of vegetation cover were the determinative factors which shaped bacterial community structures, mainly at the level of phylum *Acidobacteriota*. In salt-affected soils with either high sand content or with patchy vegetation cover, members of the classes *Blastocatellia* and *Vicinamibacteria* were the abundant acidobacteria, while in the slightly disturbed meadow soil having higher clay content, members of the class *Acidobacteriia* overwhelmingly dominated the acidobacterial community.

5. NEW SCIENTIFIC RESULTS

1. The findings of the research indicate that abiotic properties account for over 86% of the variation in microbiological properties. The driving factors vary depending on the specific location, with different factors observed in Hungary and India. In Hungary, soil organic carbon, moisture, phosphorus (P_2O_5), and potassium (K_2O) were identified as the main driving factors, while in India, pH, electrical conductivity (EC), available magnesium (avMg), and available calcium (avCa) had the greatest impact on soil microbiological properties.
2. Results of microbial activity and community structure (PLFA) showed that soil types/reference groups were the main driving factor as salt-affected soils and slightly salt-affected soils are far away from each other in terms of soil classification taxonomic distances, for soil groups with short soil classification taxonomic distances, land use had more pronounced effects on soil microbiological properties than the soil chemical and physical properties.
3. Results of the bacterial community analysis have shown that besides land use and land cover, soil texture had an important effect on the presence or lack of some bacterial classes in the communities. The presence of *Acidobacteriota* phylum was mainly determined by soil texture. It was observed that the *Acidobacteriia* class was predominantly abundant in clayey textured Solonetz meadow soils and were marginal in the other samples. However, members of the class *Blastocatellia* were highly abundant in the sandy soil textured sites viz. Szappanszek and Apaj.
4. Similarly, class *Actinobacteria* and *Thermoleophilia* of phylum Actinobacteriota were highly abundant in both Solonetz meadow and Solonchak pasture soils and were marginal in other samples. The relative abundance of both classes was higher in Solonetz meadow comparative to Solonchak pasture. However, the higher abundance of Actinobacteriota at Apaj site was characterized by the sandy soil texture and high sodium content.

5. Despite low enzyme activities detected in Gleysol pasture soils as compared to other salt-affected soils, the abundance of *Bacilli* within Phylum Bacillota were remained notably high (more than 9%). This observation highlights the unique adaptability of *Bacilli* in distinct soil habitats.
6. Arable fields with regular soil tillage had the highest rates of shared OTUs while non-disturbed meadow and pasture sites showed higher variability. Cultivation was the main driving factor in shaping the bacterial diversity in arable lands characterized with different soil types.
7. The results showed that the OTUs belonging to class *Ktedonobacteria* reached a relatively high abundance of more than 9% in Solonetz meadow soil, which is similar to what was reported only in geothermal sediments.
8. Despite rigorous investigation, none of the investigated environmental parameters could explain the outlying nature of NSnM. This intriguing observation suggests the presence of underlying factors beyond those traditionally considered in soil ecology, emphasising the impact of unknown variables or complex interactions within the soil microbiome.
9. In case of the members of the class *Vicinamibacteria* the high amount of nutrients originated from fertilizers in an arable soil seems to be stronger environmental factor than plant covering of grasslands.
10. Although classical and molecular microbiological techniques, such as PLFA and DNA analysis, as well as traditional microbiological methods, have offered valuable information about the soil microbial community and its activity, my research highlights the importance of using a comprehensive approach to fully understand and characterize the soil microbiological status. The results of my study show that the use of innovative research methods has a significant impact on the findings, emphasizing the need for a comprehensive methodology to gain a detailed understanding of soil microbiology.

6. RELATED PUBLICATIONS

RESEARCH ARTICLE

R.K. Gangwar, A. Táncsics, M. Makádi, M. Farkas, M. Cserháti, E. Michéli, M. Fuchs & T. Szegi. 2024. Comparative bacterial community analysis of Hungarian salt-affected soils: effects of different land usage on the community composition. Biologia Futura. (Accepted) (IF = 2.1) (Q1)

R.K. Gangwar, M. Makadi, B. Bresilla, M. Zain, T.G. Weldmichael, I. Demeter, A. Tancsics, M. Cserhati, T. Szegi. 2022. Effects of land uses and soil types on microbial activity and community structure. International Agrophysics, 36(4), 323-336. https://doi.org/10.31545/intagr/155096 (IF = 2.2) (Q1)

R. K. Gangwar, M. Makádi, I. Demeter, A. Táncsics, M. Cserháti, G. Várbíró, J. Singh, Á. Csorba, M. Fuchs, E. Michéli & T. Szegi. 2021. Comparing Soil Chemical and Biological Properties of Salt Affected Soils under Different Land Use Practices in Hungary and India. Eurasian Soil Science, 54(7), 1007-1018. https://doi.org/10.1134/S1064229321070048 (IF = 1.4) (Q2)

T. G. Weldmichael, T. Szegi, L. Denish, **R. K. Gangwar**, E. Michéli, B. Simon. 2020. The patterns of soil microbial respiration and earthworm communities as influenced by soil and land-use type in selected soils of Hungary. Soil Science Annual, 71(2), 139–148. (https://doi.org/10.37501/soilsa/122408) (IF = 1.5) (Q2)

R. K. Gangwar, M. Makádi, M. Fuchs, Á. Csorba, E. Michéli, I. Demeter, A. Táncsics, T. Szegi. 2019. Changes of soil microbial parameters of salt affected Solonetz soils under arable and pasture land use. Agrokémia és Talajtan, 68(1), 155-175. https://doi.org/10.1556/0088.2019.00024 (Q4)

R. K. Gangwar, M. Makádi, M. Fuchs, Á. Csorba, E. Michéli, I. Demeter, T. Szegi. 2018. Comparison of biological and chemical properties of arable and pasture Solonetz soils. Agrokémia és Talajtan (Agrochemistry and Soil Science) 67(1), 61-77. https://doi.org/10.1556/0088.2018.67.1.5 (Q4)

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R.K. Gangwar, M. Makádi, J. Singh, T. Szegi. 2021. Review of farmers land use systems and their evaluation based on chemical, physical and microbiological properties of Indian Solonetz soils. 13th International Conference on Agrophysics: Agriculture in changing climate. 15-16 November 2021, Lublin, Poland. pp 112. (Abstract)

T.G. Weldmichael, T. Szegi, L. Denish and **R.K. Gangwar**, E. Micheli, B. Simon. 2020. Significant Influence of Land Use Type on Earthworm Communities but Not on Soil Microbial Respiration in Selected Soils of Hungary. ICSBB 2020: International Conference on Soil Biology and Biochemistry, London, United Kingdom, March 12-13, 2020. (Abstract)

R.K. Gangwar, M. Makádi, E. Michéli and T. Szegi. 2019. Soil salinization-a serious environmental threat: with reference to Indian salt affected soils. International seminar on "Environmental Issues and Challenges in the 21st Century" (EICC-2019). Bareilly College, Bareilly, U.P. India, 20th to 22nd January 2019. (Abstract)

R.K. Gangwar, J. Singh, M. Makádi, T. Szegi. 2017. Response of microbial biomass and it's activity to seasonal changes in salt affected soils of India. International conference on Long term field experiments. Nyiregyhaza, Hungary, September 27-28, pp. 35. (Abstract)

R.K. Gangwar, M. Makádi, E. Michéli, T.G. Weldmichael and T. Szegi. 2017. Impact of soil types and management practices on soil microbiological properties - a case study in salt affected area of Hungary. European Geosciences Union-General Assembly 2017. Vienna, Austria, 23–28 April, Geophysical Research Abstracts, Vol. 19, EGU2017-16601. (Abstract)

R.K. Gangwar, J. Singh, M. Marianna, M. Erika and S. Tamás. 2016. Carbon dioxide emission related to microbial biomass of salt affected soils. International seminar on "Recent Trends and Experimental Approaches in Science, Technology and Nature". IISR, Lucknow, India, 23rd - 24th December 2016. (ISBN- 978-81-932601-6-6) (Conference paper)

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