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

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## LIST OF ABBREVIATIONS

AMF	Arbuscular Mycorrhiza Fungi
ANOSIM	Analysis of Similarity
ANOVA	Analyses of Variance
AScP	Apaj Solonchaks Pasture Land
BaCO <sub>3</sub>	Barium carbonate
BSR	Basal Soil Respiration
C:N	Carbon to Nitrogen ratio
CCA	Canonical Correspondence Analysis
CEC	Cation Exchange Capacity
CHCl <sub>3</sub>	Chloroform
CO <sub>2</sub>	Carbon Dioxide
DHA	Dehydrogenase Activity
DNA	Deoxyribonucleic Acid
EC	Electrical Conductivity
ESP	Exchangeable Sodium Percentage
FAO	Food and Agriculture Organization
GC-MS	Gas Chromatograph-Mass Spectrometer
HCl	Hydrochloric Acid
HSD	Honestly Significant Difference
IGP	Indo-Gangetic Plains
IUSS	International Union of Soil Science
K <sub>2</sub> SO <sub>4</sub>	Potassium sulfate
KSnA	Kittauna Solonetz Arable Land
KSnB	Kittauna Solonetz Bare Land
KSnP	Kittauna Solonetz Pasture Land
MBC	Microbial Biomass Carbon

NaOH	Sodium hydroxide
NCBI	National Centre for Biotechnology Information
NChA	Nádudvar Chernozem Arable Land
NSnA	Nádudvar Solonetz Arable Land
NSnM	Nádudvar Solonetz Meadow Land
OC	Organic Carbon
OTU	Operational Taxonomic Unit
PAST	Paleontological Statistics
PCA	Principal Component Analyses
PLFA	Phospholipid Fatty Acids
rRNA	Ribosomal Ribonucleic Acid
SAR	Sodium Adsorption Ratio
SAS	Salt-Affected Soils
SGIP	Szappanszék Gleysol Pasture Land
SOM	Soil Organic Matter
SPSS	Statistical Package for the Social Sciences
TPF	Triphenylformazan
TTC	Triphenyl tetrazolium chloride
UNESCO	United Nations Educational, Scientific and Cultural Organization
UPGMA	Unweighted Pair Group Method with Arithmetic mean
USDA	United State Department of Agriculture
WRB	World Reference Base for Soil Resources

# 1. INTRODUCTION

## 1.1 Background

Salt-affected soils (SAS) are widely distributed throughout the world. Mostly, occur in arid and semi-arid regions but also found in some humid to sub-humid climatic areas, particularly in the coastal regions where the inward movement of sea water through estuaries and rivers and through groundwater causes large-scale salinization (Abrol et al., 1988). According to Food and Agricultural Organization (FAO) report, over 6% of the world's total geographic area are covered with salt-affected soils which are saline and sodic and cover over 400-million-hectare (Mha) lands of topsoil (0-30 cm) and over 800 Mha of subsoil (30-100 cm) (FAO, 2022). Of the current 230 Mha of irrigated land, 45 Mha is salt-affected (19.5 %) (Arora, 2017). One to three Mha of salt-affected soils were estimated in Europe by Joint Research Centre Institute for Environment and Sustainability (European Commission 2007). Whereas in Hungary, approximately 10% of the total geographical area is covered with salt-affected soils (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).

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). Nutrient availability in soils were also affected adversely with high concentrations of salts in soils causing pseudo nutrient deficiency in soil water to crop (Singh et al., 2016). An increase in soil salinity and sodicity result in less microbial and plant growth, and salinity beyond tolerance may cause death of microbial cells and degradations of plant tissues which may also affects the biodiversity and carbon storage (Rietz and Hynes 2003; Sardinha et al. 2003).

High soil salinity and sodicity are a major limiting factors of crop yield in these areas, since many crop species are very sensitive to soil salinity and sodicity (e.g., glycophytes). Plants growing in salt-affected soils have to cope with multiple stress factors, including ionic and drought stress (Zhu, 2002). The low water potential caused by high salt deposition in soil makes nutrient uptake increasingly different for plants. Besides, high concentration of sodium ions are toxic to plant

cells, causing reduced photosynthesis, increased production of reactive oxygen species, growth inhibition or even plant death (Tuteja, 2007).

## **1.2 Significance of research**

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). Reclaiming such soils is a challenging and costly endeavor. 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). Several authors reported the effects of changes / variations in electrical conductivity (EC) and exchangeable sodium percentage (ESP) or sodium adsorption ratio (SAR) on soil microbial activities (Rietz and Haynes, 2003; Tripathi et al. 2006; Setia et al. 2011; Iwai et al., 2012). Rietz and Haynes (2003) reported that the fluctuations in microbial activities in saline and sodic soils are possibly due to direct toxic effects of salts on microbial communities. Furthermore, Wong et al. (2010) reported the toxic effects of salinity on vegetation (crop, grass and tree), resulting decreased organic matter inputs (like crop residue, litter, and fine roots) in the soil and, which in turn, significantly decreased microbial activities (Singh et al. 2014a).

Several studies suggested that there is a strong interaction between land use and soil properties as land use practices affect the soil quality, soil functions and ecological processes due to the modification in the physical, chemical and biological properties of soils (Pouyat et al. 1995; Bending et al. 2002; Balota et al. 2003; Bardgett et al., 2014; Wu et al., 2020). Several management strategies have been implemented over the years other than the inherited sustainable use of the land and over management/utilization or abandonment of land occur as a result of these practices (Pinto-Correia and Mascarenhas 1999). These land-use practices are leading to a modification of the ecosystem, which may result in soil degradation (Costa et al., 2013).

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.

### **1.3 Study objectives**

Based on the research problem statement that significant differences in terms of chemical and microbiological properties would be observed due to the land use systems, following objectives were identified:

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. LITERATURE REVIEW

In this chapter, an overview of soils of Hungary and India is presented. Further a brief description about salt-affected soils (SAS), their extent and problems related to SAS are discussed. Physical, chemical and microbiological properties of soil play an important role in different soil functions (plant growth and soil fertility). Hence, these soil properties were discussed in detail. Lastly, effect of land use and different soil types on soil properties are discussed in detail.

Soils are the foundation of terrestrial ecosystems and are essential for supporting plant growth and providing a habitat for a wide range of organisms. It is a complex mixture of minerals, organic matter, air, water, and organisms that has taken thousands of years to form. Soil is the primary medium for crop growth and is essential for producing food to feed the world's growing population (Schulte et al., 2014). Also, the soil is a major reservoir for biodiversity, over one-fourth of all living species on Earth are strict soil or litter dwellers (Decaëns et al., 2006). The majority of soil biomass is formed by microorganisms, such as algae, fungi and bacteria and archaea. One teaspoon of soil contains several thousands of microbial species, several hundred meters of fungal hyphae, and more than one million individuals (Schaefer and Schauer mann 1990; Wardle et al., 2004). Despite having importance in terrestrial ecosystem services, various strategic reports have highlighted other critical concerns for sustainable development on food security, freshwater and energy accessibility, climate change, and biodiversity loss as the primary issues, rather than soil degradation (Bouma 2014).

Soil quality deterioration is a significant problem globally, affecting agricultural productivity and food security. This problem is exacerbated by the increase in soil salinity caused by high salt content. When soils become salt affected, it adversely affects crop productivity, agricultural sustainability, and soil biomass (Suarez, 2001; Pitman and Läuchli, 2002; Tanji and Wallender, 2011). High levels of salt in soil can cause plant roots to have difficulty absorbing water and essential nutrients, leading to stunted growth and reduced crop yields (Hailu and Mehari, 2021). This can cause a significant impact on agricultural sustainability and food security.

Salt-affected soils (SAS) is a term that describes saline soils, sodic soils and saline-sodic soils. Saline soils contain salts more soluble than gypsum in a concentration sufficient to negatively affect the ability of plants to take up water. These soils have a high electrical conductivity from a saturation extract ( $EC_e$ )  $> 4 \text{ dS m}^{-1}$ ,  $pH < 8.5$  and exchangeable sodium percentage (ESP)  $< 15$ . While sodic soils contain high amounts of sodium ions that weaken the bond between the soil particles forming the soil's structure having  $EC_e < 4 \text{ dS m}^{-1}$ ,  $pH > 8.5$  and  $ESP > 15$ . Whereas saline-sodic soils are defined by high ESP levels ( $> 15$ ) with high electrical conductivity ( $EC_e > 4$

dS m<sup>-1</sup>). In these soils, both soluble salts and exchangeable Na<sup>+</sup> are high. Since electrolyte concentration is high, the soil pH is usually <8.5 and the soil is flocculated (Shaygan and Baumgarti, 2022; Sparks et al., 2024).

Eight percent of the world's land surface is covered by salt-affected soils (Szabolcs, 1979) which are usually found in arid and semi-arid regions (Pandey et al., 2011; Ferreira et al., 2016) but covering other various regions as well (Lambers, 2003; Shaygan and Baumgarti, 2022). Soil salinization and sodification are among the most common soil degradation processes threatening soil resources globally (Singh, 2016). On a worldwide level, every minute, there is a loss of productivity of 3 hectares of arable land due to secondary salinization, resulting in 10-20 million hectares of irrigated land becoming unproductive annually (Bridges and Oldeman, 1999).

Various techniques are commonly employed to ameliorate salinity and sodicity in salt-affected soils, such as leaching, soil amendment application (gypsum, farmyard manure, pyrites etc.), halophyte revegetation, and salt scrapping. However, not all reclamation methodologies are universally applicable to saline soils. Site-specific reclamation strategies must be planned, and based on understanding the soil, plant and climate interactions. In certain situations, a variety of methods may be necessary for land reclamation. This might involve using salt scrapping to eliminate salts from the top layer of soil, incorporating physical amendments to improve soil pore systems and increase salt leaching, applying chemical amendments to maintain optimal soil conditions, and finally establishing halophytes to expand the desalinization zone (Gheyi et al., 2022; Shaygan and Baumgarti, 2022; Basak et al., 2023).

Land use can have significant impacts on soil properties, including soil organic matter content, nutrient availability, soil structure and soil microbiological properties. Agricultural land use can lead to a decrease in soil organic matter content, while forested land use can increase it. Additionally, land use practices such as grazing and tillage can lead to changes in soil structure, potentially leading to reduced water infiltration and increased erosion (Mills and Fey, 2003). Changes in soil properties can have far-reaching effects on ecosystem health and productivity. Therefore, it is important to consider the impacts of land use on soil properties when making land management decisions.

## **2.1 Soils of Hungary: characteristics and distribution**

The country has a diverse geography, including the Great Hungarian Plain, the Transdanubian Hills, and the Northern Mountains. Soils in Hungary are influenced by the geology, climate, hydrology, and land use. Hungary has a diverse range of soils, with the majority being formed from the weathering of basic and acid rocks or sediments. According to Michéli et al., (2006), major soil types in Hungary are: skeletal soils, shallow soils influenced by the parent material,

brown forest soils, chernozems, salt affected soils, meadow soils, peat soils, soils of swampy forests, and soils of alluvial and slope sediments.

Chernozems are the most fertile soils in Hungary with high base saturation and thick, dark, mollic horizons. They commonly form on loess or loess-like parent material under grassland vegetation and are characterized by high biological activity (Michéli et al., 2006). They are found in the Great Hungarian Plain and are widespread soil type in Hungary. Solonchaks are strongly saline soils with high concentration of “soluble salts”. They develop in areas where evapotranspiration is considerably higher than the precipitation (Michéli et al., 2006). These soils have developed in the Danube-Tisza interfluvium region which is the largest region of alluvium sandy soil in Hungary (Kozak and Egerszegi, 1974). Solonetz soils are found in areas with high salinity levels and poor drainage. They are characterized by a hard, impermeable layer of clay and a high concentration of sodium ions. These soils are the typical sodic soils on the Great Hungarian Plain, mostly east of the Tisza River, but also west of the Danube River (Tóth, 2010). Gleysol soils are characterized by their poor drainage and waterlogging, which leads to the formation of a grayish-blue color due to the reduced iron compounds. They are typically found in low-lying areas, such as floodplains and wetlands, and are common along the Danube River in Hungary (Michéli et al., 2022).

Despite their diversity, many of Hungary's soils face challenges such as erosion, nutrient depletion, and pollution. Land use changes, climate change, and agricultural intensification are all factors that can affect soil health and productivity. Moreover, soil degradation is still a major concern in Hungary, particularly in areas with intensive agricultural practices (Michéli et al., 2022).

## **2.2 Soils of India: characteristics and distribution**

India is a country with diverse soils, which can be broadly classified into alluvial, black, red, laterite, and desert soils. The soils of India have been the subject of numerous studies over the years, and a vast body of literature exists on the topic. The earliest investigations on soils of India were done by Voelcker (1893) and by Leather (1898). They categorized the soils of the country into four major groups, namely the Indo-Gangetic alluvium, the black cotton soil or regur, red soil and laterite soil. Later, in 1995, Wadia and Korisetar compiled a soil map of India with emphasis on geological formations and classified the soils as red, black (regur), laterite and lateritic soils of Peninsular India, delta, desert, bhabar, terai and alkali soils of the Indo-Gangetic Plains (IGP). Later a revised soil map of India was generated with 23 major soil groups under FAO/UNESCO's scheme on World Soil Map project. This map was refined with 25 broad soil classes represented on a 1:7 million scale map (Govinda Rajan, 1971). The major soil types and their extent are given in Table 1.



**Table 1:** Major soil types and their extent in India

Major soils (Traditional name)	Extent (%)	Distribution in states	Soil orders US soil taxonomy
Alluvial	33.5	J&K, HP, Punjab, Haryana, Delhi, UP, Gujarat, Goa, MP, MS, AP, Karnataka, TN, Kerala, Puducherry, Bihar, Odisha, WB, ArP, Assam, Nagaland, Manipur, Mizoram, Tripura, Meghalaya, A&N	Inceptisols, Entisols, Alfisols, Aridisols
Red	26.8	AP, Karnataka, Kerala, TN, Puducherry, Rajasthan, MP, MS, Gujarat, Goa, ArP, Assam, Manipur, Meghalaya, Nagaland, Mizoram, Tripura, Delhi, UP, HP, A&N	Alfisols, Ultisols, Entisols, Inceptisols, Mollisols, Aridisols
Laterites	5.5	AP, Karnataka, Kerala, TN, Puducherry, MS, Odisha, WB	Alfisols, Ultisols, Inceptisols
Black	16.6	MP, MS, Rajasthan, Puducherry, TN, UP, Bihar, Odisha, AP, Gujarat	Vertisols, Mollisols, Inceptisols, Entisols, Aridisols
Desert	8	Rajasthan, Gujarat, Haryana, Punjab	Aridisols, Inceptisols, Entisols
Others	9.6	-	-
MP, Madhya Pradesh; MS, Maharashtra; UP, Uttar Pradesh; J&K, Jammu and Kashmir; TN, Tamil Nadu; AP, Andhra Pradesh; ArP, Arunachal Pradesh; WB, West Bengal; HP, Himachal Pradesh; A&N, Andaman and Nicobar Islands.			
Adapted from Bhattacharyya et al., 2013			

### 2.3 Soil salinization and sodification

Climate, natural drainage, topography, relief position, geology, source material, and proximity to the sea are the primary natural factors that contribute to soil salinization (Akça et al., 2020). In relation to human-caused factors, the process of soil salinization has been greatly expedited due to inappropriate irrigation techniques, inadequate drainage systems, and improper land management practices (Zhou et al., 2013). According to Liu et al. (2019), the levels of primary and secondary salinization are still rising, with an estimated yearly growth of about 10% as reported by Machado and Serralheiro (2017). Abdelaziz et al. (2019) stated that more than 50% of arable land is expected to become salinized by 2050. This will cause a significant decline in soil fertility, vegetation cover, and biodiversity, as noted by Farifteh et al. (2006) and Gorji et al. (2017). These changes will affect the ecological functions of the soil and ultimately lead to its degradation and desertification, as pointed out by Peng et al. (2019).

According to our current knowledge, salt-affected soils cover around 20% of agricultural lands globally (Wang et al., 2020), which is a threat to agriculture and also for humans. These lands mostly occur in arid and semi-arid regions but can be also found in some humid to sub-humid climatic areas as well, particularly in the coastal regions where the inward movement of sea water through estuaries and rivers and through groundwater causes large-scale salinization (Abrol et al., 1988). Physico-chemical, biological, and biochemical properties of soil are affected by the salinization and sodification (Rietz and Haynes, 2003; Singh et al., 2013a) and can be responsible for major problems related to crop productivity to a significant extent (Gill et al., 2009). Crop yield in areas with high soil salinity is greatly limited due to the sensitivity of many crop species to soil salinity. Multiple stress factors, including ionic and drought stress, are faced by plants growing in salt-affected soils (Zhu, 2002). Nutrient uptake for plants becomes increasingly difficult due to the low water potential caused by high salt deposition in soil. In addition, the high concentration of sodium ions is toxic to plant cells, resulting in reduced photosynthesis, increased production of reactive oxygen species, growth inhibition, or even plant death (Tuteja, 2007; Akbarimoghaddam et al., 2011; Hailu and Mehari, 2021). Although plants have developed several traits to adapt to high soil salinity (e.g., the net exclusion of toxic ions from the shoot, or compartmentalization of toxic ions into specific tissues), rhizosphere microorganisms are also key players of the adaptation process by alleviating the abiotic stress effects (Negrão et al., 2017; Khan et al., 2021). Bacterial endophytes, which contain 1-aminocyclopropane-1-carboxylate (ACC) deaminase were shown to possess several beneficial effects on plants affected by high salinity (e.g., higher fresh and dry biomass, higher chlorophyll contents, and a greater number of flowers etc.) (Ali et al., 2014). Similarly, rhizobacteria producing osmolytes, siderophores and antioxidant enzymes may also contribute to improved salt tolerance of plants (Negrão et al., 2017). Yadav et al., (2017) found that arbuscular mycorrhizal fungi have the potential to enhance plant growth in saline environments. Thus, the knowledge of the nature of salt-affected soils and their distribution, and the understanding of the processes that lead to soil salinization should not be underestimated.

### **2.2.1 Salt-affected soils in Hungary**

Salt-affected soils are one of the most characteristic soil formations in the Carpathian Basin. These soils are characterized by high levels of salt, which can negatively impact the growth of plants and crops. In Hungary, these soils cover approximately 10% of the total land area, which is a relatively high value compared to other regions (Szabolcs & Várallyay, 1978).

Traditionally, salt-affected soils in Hungary were used for meadows or pastures due to their limited agricultural potential. However, from the early 1950s, some of these lands were converted to crop lands in an attempt to increase agricultural productivity. This shift in land use was not without its

challenges, as the high salt levels in the soil can make it difficult for crops to grow and thrive (Jassó et al., 1989).

The literature mentions that there are large areas of naturally saline and sodic soils, which means that these soils have high levels of salt and/or sodium. This can occur due to various factors such as climate, topography, and geological conditions. However, secondary salinization is known to occur, which refers to the process of non-saline soils becoming saline due to human activities such as irrigation, mining, and urbanization. The geological and hydrological conditions of the country are such that it demonstrates the most characteristic features of natural continental (not marine) salinization, sodification and alkalinization (Tóth, 2010).

### **2.2.2 Salt-affected soils in India**

In India, 6.73 million hectares (Mha) of salt-affected soils are estimated among others cover some parts of the Gangetic plain of Uttar Pradesh; the arid and semiarid regions of Gujarat and the peninsular plains of Maharashtra state (Mandal et al., 2009). By 2050, the areas under salt-affected soils are estimated to increase from 6.74 to 16.2 Mha, unless step towards proper soil and land management practices are taken (Vision, 2015), i.e., an increase from 5 to 11% of total net sown area of the country which may turn large areas of crop lands to completely barren (Mandal et al., 2018). The total geographical area of Uttar Pradesh is 24.1 Mha, and approximately 1.37 Mha land has been affected by soil salinization and sodification which is more than 5.5 % of the total area. More than half million-hectare abandoned or fallow land due to different soil degradation processes originated from the mismanagement (Mandal et al., 2009; ISFR, 2011; Arora, 2017). Salinization and sodification has become severe threat in Indo Gangetic plains of Uttar Pradesh.

There are many problems associated with soil salinization 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 damaging effects of soil salinity and alkalinity only become apparent when the process reaches from moderate to extreme state (Wijnja and Bruggenwert, 1994) with the reclamation of these soils being both difficult and expensive.

### **2.3 Effect of land use and management on soil properties**

There is a close interaction between land use and soil properties as land use practices affect the soil quality, soil functions and ecological processes due to modifications in the physical, chemical and biological properties of the soils. For example, Pouyat et al. (1995) described that the magnitude of difference between urban and rural land use types was great for many soil chemical properties. Jaiyeoba (1995) observed that different land uses were less efficient than the natural

savannah in protecting the soil from loss of organic matter and nutrients by offtake or surface washing. Another study by Islam and Weil (2000) demonstrated the deterioration of soil properties compared to soils under well-stocked natural forest which resulted from increased disruption of macroaggregates, reductions in microbial biomass, and loss of labile organic matter. Similarly, Balota et al. (2003) indicated in his study that tillage or crop rotation affect microbial immobilization of soil nutrients. The larger amount of C immobilized in microbial biomass suggests that soil organic matter under no tillage systems provides higher levels of more labile C than conventional tillage systems.

Moreover, it was evident that the immense diversity of microorganisms and animals that live belowground contributes significantly to shaping aboveground biodiversity and the functioning of terrestrial ecosystems (Bardgett and Putten, 2014). Wu et al. (2019) suggested that altered land use patterns-initiated changes in the chemical properties of the soils, which affected the composition of microbial communities in the studied area. In addition, land use change significantly affects climatic, edaphic conditions and soil microbial attributes in terrestrial ecosystems. Total phospholipid fatty acids (PLFAs), fungi, bacteria and Actinobacteria biomass were largely decreased in the land which are converted to cropland (Chen et al., 2022).

Soil quality is determined by its physical, chemical, and biological characteristics, as it is an active component of an ever-changing environment (García-Ruiz et al., 2008). Alterations in land use are closely linked to modifications in soil quality, which can be observed through changes in specific physicochemical properties and microbial indicators (Aon and Colaneri, 2001). Changes in land-use types cause modifications in soil chemical properties. These changes have a significant impact on microbial indicators, including basal respiration, microbial biomass C, and mineralizable nitrogen forms. These fluctuations in microbial indicators can be considered as a potential indicator of assessing soil health, in response to varying land-use types and underground properties that are closely interconnected (Liu et al., 2018). Thus, the effect of land use on physicochemical and microbiological properties are discussed below.

### **2.3.1 Effect of land use and management on soil physical and chemical properties**

Land use can have a significant impact on soil chemical properties, which can affect the fertility and productivity of the soil. Effects of land use on soil chemical properties have been reported by several authors (Liu et al., 2018; Zajíčová and Chuman 2019; Alawamy et al., 2022; Buraka et al., 2023). Land use practices such as agricultural cultivation, urbanization, and industrialization can affect soil pH. For example, the pH and EC of the soil surface are impacted by the increased capillary rise of saline groundwater, which is linked to higher evaporation rates (Jalili et al., 2011)

and significant differences were observed in pH and EC of different land management practices by Falkengren-Grerup et al. (2006) and Coronado et al. (2015).

Different land uses can result in different nutrient content in soils. The alteration of land use brings about a notable impact on the stock of vegetation biomass and the diversity of plant species. Consequently, this impacts the organic residue inputs and outputs, which ultimately affects the nutrient content of the soil (Yang et al., 2014, Liu et al., 2018). Falkengren-Grerup et al. (2006) studied the impacts of land use on soil nitrogen, phosphorus, carbon, and pH persist over 40–80 years of forest growth on agricultural soils and the study suggests that these land use differences have long-lasting effects on the ecosystem that should not be overlooked. Similarly, some previous studies have demonstrated that soil nutrient contents are significantly affected by land use changes e.g., in a study conducted by Ho et al. (2018), it was demonstrated that wetlands located in heavily managed, grazed, or burned pastures have the ability to sequester soil phosphorus and nitrogen. This can have a significant impact on nutrient processing in agricultural landscapes and watersheds. In another study, the influence of land use conversion on soil organic carbon, nitrogen, phosphorus, and potassium contents and stoichiometric ratios in a salt-affected region of north-eastern China was assessed by Yu et al. (2019). They studied soils from five different land use treatments, which included corn cropland, alfalfa perennial forage, monoculture *Lyemus chinensis* grassland, monoculture *Lyemus chinensis* grassland for hay, and successional regrowth grassland. The results indicated that the type of land use plays a significant role in influencing the levels and ratios of soil nutrients, and the restoration of vegetation on formerly cultivated land can enhance soil nutrients.

The degradation of soil can be attributed to significant factors such as soil salinity and sodicity. To ensure sustainable land use and maintain soil quality and health, it is important to understand the shifts in soil salinity and sodicity. These can be expected to gradually decrease with the conversion of cropland to grasslands in semi-arid agroecosystems (Yu et al., 2018). Pessoa et al. (2022) conducted a study to determine the salinity status of soil in various land-use conditions, including low salinity areas (native vegetation), areas with varying saline levels (cultivated areas), and highly saline areas (desertified by salinity) in the semi-arid region of Northeast Brazil. The study found that the contents of exchangeable and soluble elements in the soil increased significantly due to irrigated agriculture in the region. The primary elements present in the soil solution of both cultivated and desertified regions are  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Na}^+$ , and  $\text{Cl}^-$ . Therefore, it is important to implement careful soil and water management strategies to prevent degradation.

The Earth's soil resources are under immense pressure due to the growing human population's need for food and energy. This has resulted in intensive land management practices that often lead

to a reduction in the storage of soil organic carbon (SOC) (Maskell et al., 2013; Sanderman et al., 2017). The way land is used and managed determines whether the soil will release or absorb atmospheric carbon (Lal, 2004). In general, land management techniques that involve less disturbance to the soil lead to an increase in the accumulation of soil organic carbon. Guo and Gifford (2002), as well as Kasel and Bennett (2007), have shown that the conversion of natural forest ecosystems to cultivated systems results in a loss of soil organic carbon. However, Choudhury et al., (2014) has indicated that the growth of vegetation on deserted or unused agricultural land can improve its ability to store carbon. Thus, it is important to understand the soil organic carbon in relation to different land use and management practices.

### **2.3.2 Effect of land use and management on soil microbiological properties**

To examine the soil processes in various land-use systems and preserve and restore the soil's capability to provide ecosystem services, it is essential to conduct research on how human activities, like land-use change, affect soil ecosystem functioning (Van Leeuwen et al. 2017). The physicochemical properties of soil are known to remain relatively unchanged over time, as it can take several decades to observe any alterations, even after long-term land use modifications (Parr and Papendick, 1997; Arévalo-Gardini *et al.*, 2015). However, soil microbe-related indicators, such as microbial biomass, respiration, and enzyme activities, are typically reliable indicators for measuring both the quantitative and qualitative changes in microbial communities due to alterations in land use or management systems (Liu et al., 2018; Meena and Rao, 2021; Mencil et al., 2022a; Bhaduri et al., 2022).

Recently, there has been a growing interest in studying the effects of agricultural land use on the biological and biochemical characteristics of soils. The importance of microorganisms in ecosystem functioning has led to an increased interest in determining soil microbial biomass (Azam et al., 2003). The living part of soil organic matter, known as the microbial biomass, usually constitutes 1-5% of the total organic matter. Since the microbial biomass has a quick turnover rate, alterations in soil management practices can have a prompt effect on its carbon content, as stated by Gregorich et al. (1997). Kara and Bolat (2008) carried out a study that demonstrated that land use can impact the microbial biomass C in soil by changing the inherent soil characteristics within same ecological conditions.

Soil microbial communities play a fundamental role in supporting plant/crop growth by regulating nutrient cycling, organic matter decomposition and ecosystem processes that are important for the growth and maintenance of plants (Lehman et al., 2015a; Ren et al., 2018; Fanin et al., 2019). Soil environmental conditions such as physicochemical factors govern the composition and diversity of these microbial communities (Bass Becking, 1934), which are particularly important to

sustainable agriculture (Lakshmanan et al., 2014; Lehman et al., 2015b, Bender et al., 2016). Thus, developing our understanding of microbial communities and how various management practices (i.e., different land use practices) impact these communities and their diversity is of the utmost importance.

Assessing the microbial community structure in soils was difficult until the development of phospholipid fatty acid (PLFA) analysis (Bobbie and White, 1980) which is presently an increasingly popular method which may contribute to our understanding of ecosystem function and sustainable land management (Veum et al., 2019). PLFA analysis has been used in several studies which reported the impact of different land use practices on microbial community structure (Yuan et al., 2015; Guo et al., 2016; Ahmed et al., 2019; Gangwar et al., 2019; Liu et al., 2020). Ahmed et al. (2019) and Rampazzo et al. (1999) showed that soil microbial properties and enzyme activities were significantly different among the various land use types. Moreover, Yuan et al. (2015) investigated the effects of land use practices on the composition of the soil microbial community by analysing soil PLFA and found that the soil microbial community structure varied to a significant extent. A meta-analysis of various land use changes revealed that microbial attributes and their determinants were particularly affected by the types of land use changes. A decline in the soil microbial community in anthropogenic cases was driven by organic carbon, total nitrogen and the C:N ratio, while in the case of natural changes the following factors played key roles; total nitrogen, phosphorus and the C:N ratio (Chen et al., 2022). According to Moran-Rodas et al. (2022), who investigated two soil types under different land uses, the highest impact factor on the soil microbial community was particulate soil organic matter content.

Soil salinity is a dominant factor in shaping the soil microbial community structure (Li et al., 2023) and can negatively affect soil microbial activity (Borsodi et al., 2021). It was also suggested, that high salinity can lower bacterial richness and increasing salinity-sodicity decreases the overall complexity of the bacterial network in soils (Guan et al., 2021). Halotolerant and halophilic microorganisms are capable of thriving in highly salty environments (Arora, 2020). In general, it is observable, that members of the phylum *Pseudomonadota* (also known as *proteobacteria*) (contain halophilic representatives) are usually the most dominant microbial community members in sodic soils (Wang et al., 2020; Borsodi et al., 2021). Similarly, halophiles can be found in various groups of bacteria, including cyanobacteria, the *Flavobacterium*-Cytophaga branch, Spirochetes, and Actinomycetes. Within the Gram-positive bacteria lineage (*Firmicutes*), halophiles are also present in both aerobic (such as *Bacillus* and related organisms) and anaerobic branches. Most halophiles in the bacterial domain are moderate rather than extreme in terms of their salt preferences. Nevertheless, there are a small number of bacterial halophiles that share

similar salt requirements and tolerance to the archaeal halophiles found in the *Halobacteriaceae* family (Arora, 2020). Besides, members of the phyla *Bacteroidota*, *Acidobacteriota*, *Gemmatimonadota*, and *Bacillota* are also often abundant in these environments (Wang et al., 2020; Borsodi et al., 2021; Guan et al., 2021). Further, a taxonomic analysis revealed that arbuscular mycorrhizal fungi and calcium treatment increased the abundance of *Pseudomonadota* and *Bacillota* at the phylum level in saline alkali soil (Ci et al., 2021).

A major question regarding these microbial communities is that which environmental parameter(s) is/are the dominant driving force that shapes the community structure. A strong correlation between microbial community composition with edaphic factors was observed by Wang et al. (2022). Based on the microbial analyses of grassland sodic soils (Songnen Plain, China) Wang et al. (2020) suggested that electrical conductivity (EC) content of soil was the most important driving force for bacterial composition, followed by sodium ion content. Borsodi et al. (2021) investigated sodic soils covered with different kind of alkali steppe vegetation (Danube-Tisza Interfluvium, Hungary) and found that microbial catabolic profiles in the investigated soil samples were primarily driven by EC and soil water content. Besides, the only environmental variable, which significantly influenced the bacterial community structure at taxonomic level was soil  $\text{CaCO}_3$ .

Although significant new knowledge was gained in the past few years on microbial communities of salt-affected soils worldwide, still little is known on how different land usage, plant coverage, plant composition, and soil properties affect bacterial assemblages. However, such studies are needed to reveal the possible negative effects of overgrazing or irrational utilization of alkali-saline lands to avoid e.g., the loss of bacterial diversity, which can severely affect ecosystem functions (Singh et al., 2014b; Wagg et al., 2019).



### 3. MATERIALS AND METHODS

This chapter provides the details of the study area, sampling stations and the methods used in the laboratory for soil analysis. The detailed procedures for the analysis of soil physical, chemical and microbiological methods are discussed in this chapter.

#### 3.1 Study area

##### 3.1.1 Location

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 2 whereas Table 3 represents meteorological parameters of both locations.

**Table 2:** Soil reference groups and land use types of the studied sites

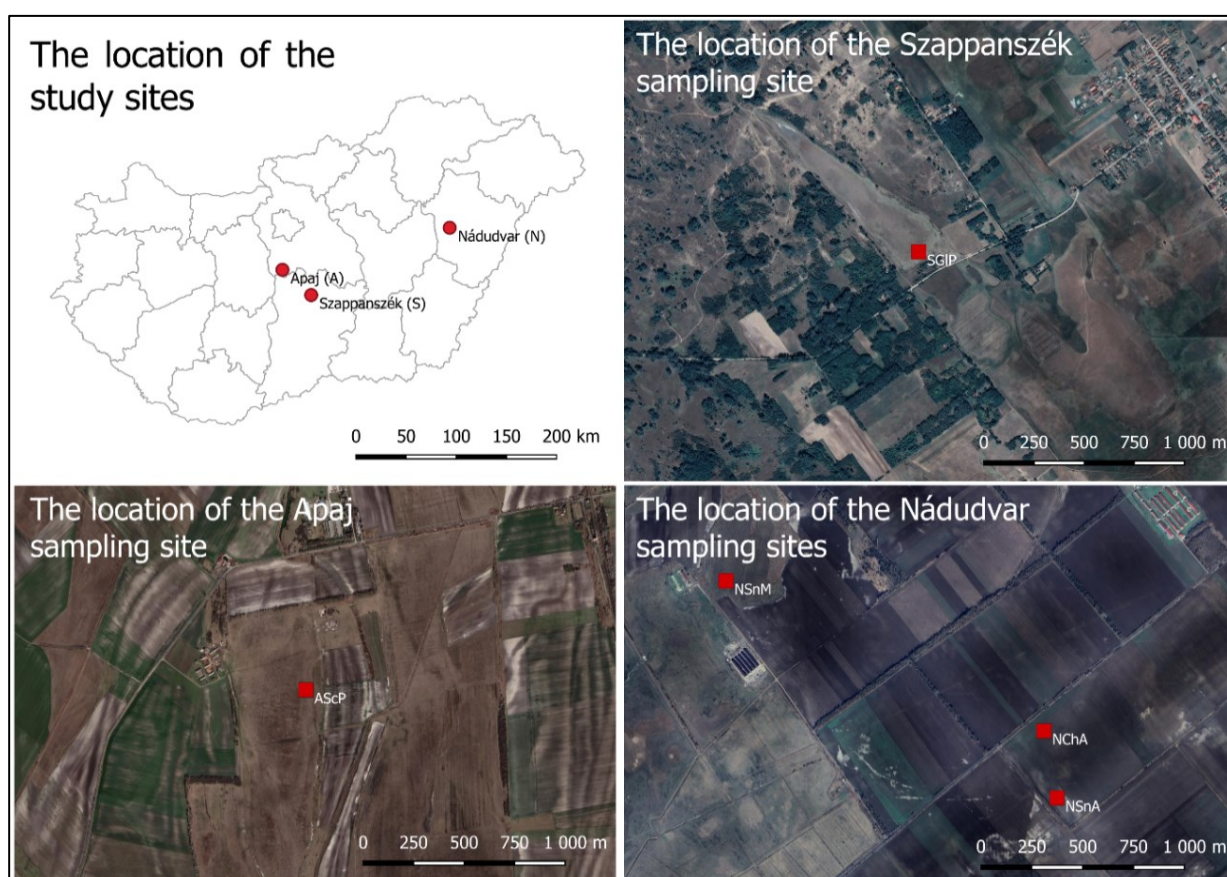
Location	Sampling Site	Soil reference group	Land use type	Abbreviation used
Hungary	Nádudvar (N)	Solonetz (Sn)	Arable (A) land	NSnA
			Meadow (M) land	NSnM
		Chernozem (Ch)	Arable (A) land	NChA
	Apaj (A)	Solonchaks (Sc)	Pasture (P) land	AScP
	Szappanszék (S)	Gleysol (Gl)	Pasture (P) land	SGlP
India	Kittauna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

**Table 3:** Climate of sampling sites in Hungary and India

Location	Climate	Sampling Site	Temperature (°C)			Annual Precipitation (mm)
			Annual	Min.	Max.	
Hungary	Typical European continental/Pannonian with warm and dry summers	Nádudvar (N)	9.8-10.2	-17	35	510 – 550
		Apaj (A)	10.3-10.5	-17	34	510 – 550
		Szappanszék (S)	10.2-10.3	-16	35	530 – 570
India	Sub-tropical	Kittauna (K)	25.1	6	40	1037

- a. **Hungary:** Soil samples were collected from Nádudvar (Hajdú-Bihar County) (NSnA– N 47°27'30.22" and E 21°11'46.04" ; NSnM – N 47°28'5.53" and E 21°10'24.47" ; NChA - N 47°27'41.07" and E 21°11'37.54"), Apaj (Pest County) (AScP – N 47° 6'21.24" and E 19°

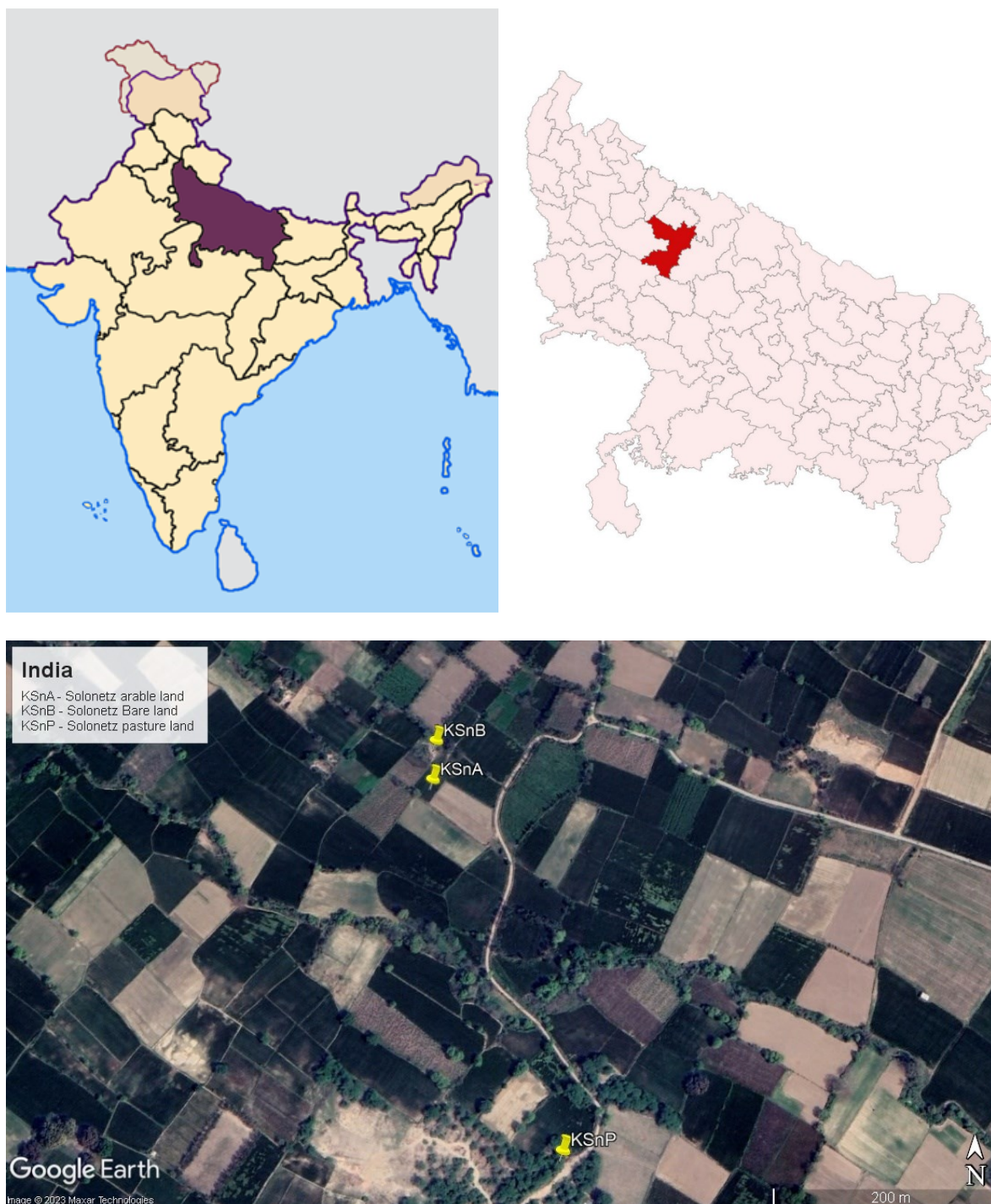
3'37.84") and Szappanszék (Bács-Kiskun county) (N 46°53'10.34" and E 19°25'33.67") in Hungary (Figure 1). The climatic conditions of all sampling sites are summarised in Table 3. The Hungarian sites encompassed three land use types viz. arable land, pasture land and meadow land. The cultivated arable sites (NSnA, NChA) were ploughed to a depth of 30 cm and 400 kg ha<sup>-1</sup> 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 (SGIP) 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 (A) land, bare (B) land and pasture (P) land was used for the study (Figure 2). The geographical coordinates of the arable land (KSnA) and bare land (KSnB) sites were latitude N–28°19'58.0" and longitude E–79°08'09.3" respectively, with an elevation of 175 m above the sea level and coordinates of the pastureland

(KSnP) site are latitude N-28°19'17.01" and longitude E-79°08'05.18". The climate of Indian site was summarized in Table 3. 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.

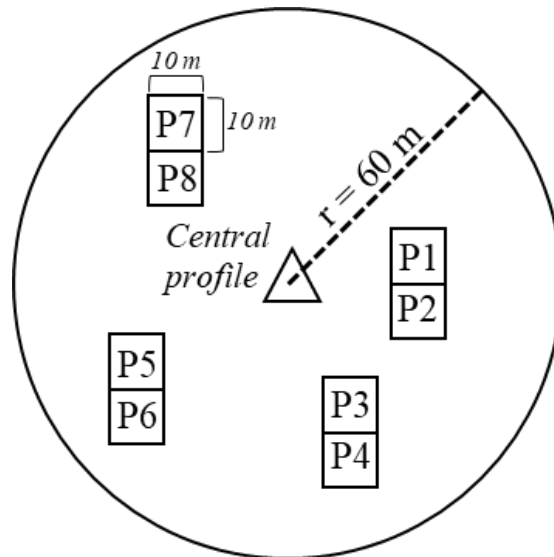


**Figure 2.** Google Earth maps showing soil sampling locations of India.

### 3.2 Soil sampling

#### 3.2.1 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<sup>2</sup> were selected from each site namely AScP, NSnA, NSnM, NChA and SGIP (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 4 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<sup>2</sup> 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 SGIP).

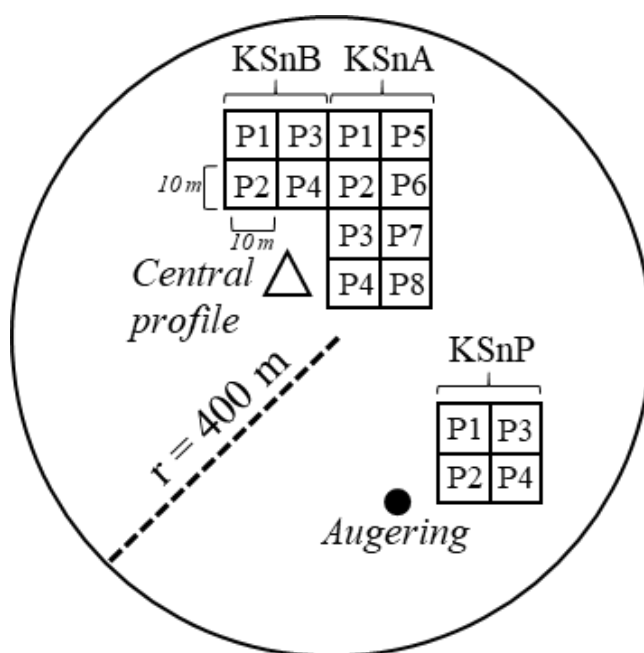
**Table 4: Parameters analysed for each sampling year**

Year	Sampling site	Samples collected	Parameters analysed	
			Physico-Chemical properties	Microbiological properties
May 2016	AScP, NSnA, NSnM, NChA and SGIP	40 samples (8 samples from each site)	OC, pH, EC P <sub>2</sub> O <sub>5</sub> K <sub>2</sub> O Mg <sup>2+</sup> Ca <sup>2+</sup> Na <sup>+</sup> Moisture	BSR MBC DHA Phosphatase
September 2016	AScP, NSnA, NSnM, NChA and SGIP	40 samples (8 samples from each site)	OC pH, EC P <sub>2</sub> O <sub>5</sub> K <sub>2</sub> O Mg <sup>2+</sup> Ca <sup>2+</sup> Na <sup>+</sup> Moisture	BSR MBC DHA Phosphatase
May 2017	AScP, NSnA, NSnM, NChA and SGIP	10 samples (2 samples from each site)	OC, pH EC P <sub>2</sub> O <sub>5</sub> K <sub>2</sub> O Mg <sup>2+</sup> Ca <sup>2+</sup> Na <sup>+</sup> Moisture	BSR MBC DHA Phosphatase PLFA properties
		5 samples (1 sample from each site)	Soil texture	Illumina 16S rRNA gene amplicon sequencing

### 3.2.2 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<sup>2</sup> 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);  $\Delta$ : location of soil profile;  $\bullet$ : location of augering

### 3.3 Laboratory analysis

#### 3.3.1 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  $exNa^+$ ,  $exCa^{2+}$ ,  $exMg^{2+}$  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<sub>3</sub> content was measured with the Scheibler gas-volumetric method (Buzás, 1988), while particle size analysis was conducted using the pipette method (Buzás, 1993).

### 3.3.2 Soil profile description

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 2 presents the soil reference groups and land use types of the studied sites. Details of site and soil profile description are found in Table 5-9 and Table 10 for sites in Hungary and India, respectively.

Laboratory data and classifications of the reference soil profiles can be found in Table 5-10.

**Table 5:** Chemical and physical properties of Nádudvar arable Solonetz reference soil profile

Master Horizon	Depth	pH <sub>H2O</sub>	CaCO <sub>3</sub>	SOM	Sand	Clay	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Na <sup>+</sup>	K <sup>+</sup>	CEC	ESP	Salt cont.	EC
	cm		%	%	%	%	cmol <sup>+</sup> kg <sup>-1</sup>				cmol <sup>+</sup> kg <sup>-1</sup>	%	%	ds m <sup>-1</sup>
Ap1	0-18	7.8	<0.1	2.18	14.56	28.78	17.7	4.0	1.1	1.5	26.8	4.2	0.07	0.66
Ap2	18-40	7.9	0.3	2.31	10.18	29.18	17.8	3.8	2.0	1.3	27.9	7.2	0.08	0.67
Bthng	40-70	8.9	0.2	2.24	13.86	27.58	18.7	5.9	9.0	0.5	35.1	25.7	0.20	1.73
2Bthng	70-100	9.2	0.6	1.15	6.17	42.91	11.4	5.4	12.0	< 0.4	30.2	39.9	0.32	2.61
3BCl	100-130	9.4	<0.1	0.57	8.81	39.81	8.9	5.1	12.2	< 0.4	27.4	44.4	0.35	2.79
3Ck1	130-150	9.5	10.2	0.46	6.96	35.71	10.9	5.2	11.9	< 0.4	28.3	42.0	0.29	2.42

Mollic SOLONETZ (Cutanic, Endoclayic, Hypernatric, Episiltic, Endoprotovetric, Bathiprotocalcic, Bathigleyic, Bathisiltic)



**Table 6:** Chemical and physical properties of Nádudvar arable Chernozem reference soil profile

Master Horizon	Depth	pH <sub>H2O</sub>	CaCO <sub>3</sub>	SOM	Sand	Clay	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Na <sup>+</sup>	K <sup>+</sup>	CEC	ESP	Salt cont.	EC
	cm		%	%	%	%	cmol <sup>+</sup> kg <sup>-1</sup>				cmol <sup>+</sup> kg <sup>-1</sup>	%	%	ds m <sup>-1</sup>
Ap1	0-25	7.9	<0.1	1.97	11.73	33.26	20.5	6.9	0.02	4.5	35.5	0	0.09	0.77
Ap2	25-40	7.9	<0.1	3.04	9.42	33.97	17.2	6.3	0.09	3.9	30.5	0.1	0.08	0.69
ABk	40-80	8.3	9.3	1.34	11.58	36.62	25.6	4.4	0.76	1.3	32.1	1.8	0.08	0.73
Bk	80-100	8.6	20.2	0.60	6.95	30.61	22.5	5.9	2.29	< 0.4	30.9	3.7	0.18	1.60
Bck	100-140	8.7	19.5	0.44	14.34	36.08	17.4	7.5	3.52	< 0.4	28.6	6.2	0.30	2.51
Ck	140-160	8.6	14.0	0.10	8.36	33.92	15.8	8.1	3.9	< 0.4	28.1	6.9	0.32	2.62

Endocalcic CHERNOZEM (Aric, Pachic, Endosodic, Pantosiltic)

**Table 7:** Chemical and physical properties of Nádudvar Meadow Solonetz reference soil profile

Master horizon	Depth	pH <sub>H2O</sub>	CaCO <sub>3</sub>	SOM	Sand	Clay	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Na <sup>+</sup>	K <sup>+</sup>	CEC	ESP	Salt cont.	EC
	cm		%	%	%	%	cmol <sup>+</sup> kg <sup>-1</sup>				cmol <sup>+</sup> kg <sup>-1</sup>	%	%	ds m <sup>-1</sup>
Oi	-2-0	na	na	na	na	na	na	na	na	na	na	Na	na	na
A	0-5	5.9	1.9	3.45	12.05	15.23	6.8	2.2	3.6	< 0.4	15.3	23.6	0.10	0.89
Btng	5-15	7.7	<0.1	0.95	7.84	42.54	7.2	5.3	13.4	0.6	30.8	43.4	0.35	2.80
Bthng	15-40	9.2	<0.1	0.84	4.96	45.07	10.3	5.2	14.5	0.7	32.6	44.5	0.61	4.09
Bthkn	40-55	9.7	18.3	0.51	7.36	40.16	9.0	6.2	21.1	0.6	37.3	56.4	0.88	5.07
Bck	55-100	10.1	19.9	0.40	5.73	35.63	11.2	4.6	18.2	< 0.4	34.3	53.2	0.90	5.16
Ck	100-120	10.2	15.2	0.23	10.23	30.96	9.8	4.8	17.5	< 0.4	32.4	54.1	0.86	4.99

na = not available

Katocalcic Katoprotosalic SOLONETZ (Epiclayic, Endosiltic, Cutanic, Differentic, Humic)





**Table 8:** Chemical and physical properties of Apaj pasture Solonchak reference soil profile

Master horizon	Depth	pH <sub>H2O</sub>	CaCO <sub>3</sub>	SOM	Sand	Clay	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Na <sup>+</sup>	K <sup>+</sup>	CEC	ESP	Salt cont.	EC
	cm		%	%	%	%	cmol <sup>+</sup> kg <sup>-1</sup>				cmol <sup>+</sup> kg <sup>-1</sup>	%	%	ds m <sup>-1</sup>
Oi	-1-0	na	na	na	na	na	na	na	na	na	na	na	na	na
Azkln	1-10	8.97	23.3	1.44	58.08	41.92	6.7	2.7	8.3	< 0.4	18.7	46.9	0.22	18.56
2Bzhln	10-30	9.75	40.6	0.53	37.63	62.37	25.3	6.8	12.8	< 0.4	45.5	28.5	0.13	11.92
3Czlh	30-60	9.75	34.7	0.33	59.76	40.24	16.8	5.9	9.5	< 0.4	33.6	29.5	0.07	7.08
3Czcr	60-100	9.45	28.3	0.57	57.35	42.65	11.1	3.9	8.8	< 0.4	24.7	36.9	0.05	5.56
3Czr	100-150	9.35	14.0	1.44	58.08	41.92	5.6	2.8	3.7	< 0.4	13.3	30.6	0.29	4.80
na = not available														

Katofluvic Anocalcic Pantosodic Amphigleyic SOLONCHAK (Alcalic, Carbonatic, Endosiltic, Bathyloamic)



**Table 9:** Chemical and physical properties of Szappanszék pasture Gleysol reference soil profile

Master horizon	Depth	pH <sub>H2O</sub>	CaCO <sub>3</sub>	SOM	Sand	Clay	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Na <sup>+</sup>	K <sup>+</sup>	CEC	ESP	Salt cont.	EC
	cm		%	%	%	%	cmol <sup>+</sup> kg <sup>-1</sup>				cmol <sup>+</sup> kg <sup>-1</sup>	%	%	ds m <sup>-1</sup>
Ak	0-5	9,67	17,35	1,48	74,87	14,09	2,71	1,87	0,02	0,22	4,82	0,4	0,08	na
Ck1	5-35	9,89	10,97	0,16	96,63	2,25	3,39	5,96	0,12	0,39	9,86	1,2	0,05	18.56
Ck2	35-50	10,03	12,77	0,13	91,19	7,05	3,48	1,78	0,07	0,20	6,05	1,3	0,11	11.92
2Clk1	50-80	10,17	27,91	0,11	85,59	8,01	18,46	6,05	0,23	0,26	25,46	0,9	0,2	7.08
3Clk2	80-100	10,12	24,99	0,11	83,35	7,69	3,78	1,77	0,11	0,20	5,98	1,9	0,19	5.56
4Crk	100-	10,09	23,19	0,1	81,43	5,45	2,71	1,87	0,02	0,22	4,82	0,4	0,18	4.80
na = not available														

Katofluvic Endocalcic Reductigleyic GLEYSOL (Alcalic, Katoarenic, Ochric, Pantosodic)

**Table 10:** Chemical and physical properties of Kittauna bare Solonetz reference soil profile

Master horizon	Depth	pH <sub>H2O</sub>	CaCO <sub>3</sub>	SOM	Sand	Clay	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Na <sup>+</sup>	K <sup>+</sup>	CEC	ESP	Salt cont.	EC
	cm		%	%	%	%	cmol <sup>+</sup> kg <sup>-1</sup>				cmol <sup>+</sup> kg <sup>-1</sup>	%	%	ds m <sup>-1</sup>
A(E)k	0-20	8.2	15.7	0.15	38	16	6.3	6.3	6.8	< 0.4	19.7	34.5	0.79	2.24
Btk	20-40	8.9	16.2	0.05	28	28	5.0	5.0	6.8	< 0.4	16.9	40.2	0.74	2.17
Bnk	40-60	9.2	14.8	na	26	11	4.3	3.4	8.1	< 0.4	16.8	48.2	0.68	1.75
2Ck	60-100	9.3	15.1	na	42	11	3.1	3.1	6.6	< 0.4	12.8	51.5	0.51	1.21
2Ck2	100-150	8.8	17.1	na	36	12	2.7	2.7	4.7	< 0.4	10.3	45.6	0.36	0.84
na = not available														

Calcic, SOLONETZ (Columnic, Cutanic, Differentic, Endoloamic, Endoraptic, Anosiltic)





### 3.3.3 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 °C with ethanol free  $\text{CHCl}_3$  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  $\text{K}_2\text{SO}_4$  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  $\text{CO}_2$ . After 10 days, the conical was removed and excess  $\text{BaCl}_2$  was added in the NaOH solution to precipitate the trapped  $\text{CO}_2$  as insoluble  $\text{BaCO}_3$ . 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  $\text{mg CO}_2 \text{ kg}^{-1} \text{ hr}^{-1}$ .

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<sup>-1</sup> dry sample.

### **3.3.4 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 -20°C 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.

### **3.3.5 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](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.

### 3.4 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.

## **4. RESULTS AND DISCUSSION**

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

#### **4.1.1 Effect of land use on soil chemical and physical properties**

The differences in soil properties (Table 11) between Hungarian and Indian soils can be explained by the differences in soil age, bedrock, climate and cropping history. In Indian soils, OC was the highest in case of arable land (KSnA) with an average of 0.33 %, intermediate in KSnB (0.25 %) and the lowest in KSnP (0.22%) while in case of Hungarian soils, OC was the highest at NSnM (5.51 %), and the lowest at NSnA (3.83 %). The low soil OC content at Indian sites in the present study agrees with the study of Rao and Pathak (1996) who reported 0.18 to 0.78% of organic carbon in salt-affected soils in India. Whereas in Hungarian soils OC was observed higher due to non-ploughing and continuous plant coverage which results in higher organic matter content in the pasture lands and leads to increased microbial activity in salt-affected soils (Tejada et al., 2006), in case of the Hungarian arable land, preferable soil and climatic conditions, or management practices can result in higher organic matter content.

Where salinization and sodification of soils is occurring in the field, soil C stores are most likely become depleted and organic matter becomes solubilised, providing additional substrate for the microbial population, while plant inputs decrease due to stresses caused by increasing salt content, induced ion toxicities and deficiencies, and declines in soil physical conditions. As this process continues, SOC can be rapidly depleted as mineralisation of SOM increases and inputs of C decrease (Wong et al., 2008).

Generally, land use change from native vegetation to cropping and continuous cropping decreases the organic carbon content of soils (Dalal et al., 2005; Guo and Gifford, 2002; Sparling 1992). According to Houghton (2010) a global average of 25–30% SOC loss is considered a conservative estimate when soil under native vegetation or permanent pasture is brought under cultivation for cropping.

Conversion of cropland to grassland is one of the most effective strategies for C sequestration (Chen et al., 2009; Lal et al., 1999; Smith et al., 2000). However, in Indian sites the arable land does not show any significant losses of OC comparing to the pasture land, but represents the highest values, as the farmers leave the crop residues after harvesting and mix it well with the top soil by ploughing.

**Table 11:** Descriptive statistics (mean and standard deviation) of soil chemical properties, moisture content and results of the Tukey's test from two sampling seasons of year 2016 samples.

		Hungary			India		
		<b>AScP (n=8)</b>	<b>NSnA(n=8)</b>	<b>NSnM (n=8)</b>	<b>KSnB (n=4)</b>	<b>KSnP (n=4)</b>	<b>KSnA (n=8)</b>
<b>Parameter</b>	<b>Unit</b>	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
<b>OC</b>	(%)	4.97 (0.60)Cb	3.83 (0.38)Ba	5.52 (0.63)Dc	0.25 (0.11)Aa	0.22 (0.11)Aa	0.33 (0.05)Ab
<b>pH</b>		7.83 (0.26)Cc	6.75 (0.55)Bb	5.54 (0.35)Aa	8.71 (0.57)Db	8.95 (0.61)Db	8.06 (0.43)Ca
<b>EC</b>	( $\mu\text{S cm}^{-1}$ )	402.21 (46.73)Bc	358.42 (65.18)Abb	224.65 (67.70)Aa	2041.33 (429.25)Dc	829.50 (333.26)Cb	274.87 (108.53)ABa
<b>AL-P<sub>2</sub>O<sub>5</sub></b>	(mg kg <sup>-1</sup> )	40.55 (10.66)Aa	671.71 (273.88)Cc	233.67 (69.99)Bb	6.86 (0.37)Ab	7.02 (0.42)Ab	4.15 (1.88)Aa
<b>AL-K<sub>2</sub>O</b>	(mg kg <sup>-1</sup> )	163.21 (16.19)Ca	383.42 (59.32)Db	177.25 (62.51)Ca	106.14 (20.98)ABc	119.04 (10.34)Bb	79.64 (7.90)Aa
<b>avMg<sup>2+</sup></b>	(mg kg <sup>-1</sup> )	40.64 (7.15)Ab	44.07 (9.81)Ab	32.63 (4.13)Aa	2126.67 (630.40)Ba	2910.00 (557.69)Cb	2325.00 (272.95)Ba
<b>avCa<sup>2+</sup></b>	(mg kg <sup>-1</sup> )	406.88 (79.02)Aa	1075.63 (216.56)Bc	606.38 (99.17)ABb	3597.33 (1129.17)Ca	4865.00 (1003.06)Db	3915.75 (415.39)Ca
<b>avNa<sup>+</sup></b>	(mg kg <sup>-1</sup> )	907.63 (207.73)Bb	288.83 (181.74)Aa	279.83 (130.49)Aa	947.00 (274.68)Bb	868.25 (106.73)Bb	288.83 (181.74)Aa
<b>Mois</b>	(%)	27.61 (6.10)Cb	21.72 (0.89)Ba	21.27 (1.69)Ba	5.97 (1.37)Aa	7.18 (0.92)Ab	7.07 (1.02)Ab
<p><i>a-c small letters indicate significant differences of means within locations between sites according to the Tukey's test (<math>p &lt; 0.05</math>)</i></p> <p><i>A-D capital letters indicate significant differences of means within all sites of both countries according to the Tukey's test (<math>p &lt; 0.05</math>)</i></p> <p>Where, OC-organic carbon; EC- electrical conductivity; AL-P<sub>2</sub>O<sub>5</sub>- available phosphorus; AL-K<sub>2</sub>O- available potassium; avMg<sup>2+</sup>- available magnesium; avCa<sup>2+</sup> - available calcium; avNa<sup>+</sup>- available sodium; Mois.- moisture content</p> <p>(Hungary: AScP = Solonchak pasture land; NSnA = Solonetz arable land; NSnM = Solonetz pasture land</p> <p>India: KSnA = Solonetz arable land; KSnB = Solonetz bare land; KSnP = Solonetz pasture land)</p>							



Soil pH values were in between slightly alkaline to alkaline (ranging from 8.06 to 8.95) in Indian salt-affected soils (Rao and Pathak, 1996) as the result of salt accumulation close to the soil surface, the highest pH was measured in Indian pasture land (KSnP) and bare land (KSnB), while in Hungary from slightly acidic to slightly alkaline (ranging pH from 5.54 to 7.84), lowest values were measured in Solonetz pasture (NSnM), and the highest values belonged to the Solonchaks pasture land (AScP). The EC values were the highest in Indian bare land (KSnB; 2041.33  $\mu\text{S cm}^{-1}$ ) and pasture land (KSnP; 829.50  $\mu\text{S cm}^{-1}$ ), followed by the Hungarian Solonchaks pasture (AScP; 402.21  $\mu\text{S cm}^{-1}$ ), the lowest EC values were measured in case of Solonetz pasture land (NSnM; 224.65  $\mu\text{S cm}^{-1}$ ) (Table 11). These results can be explained by different soil forming processes, and management practices or plant coverage as well (Gangwar et al., 2021). In the Carpathian-basin Solonchaks can be found in low-lying areas, where the groundwater table contains high amount of water-soluble salts and situated close (within 1 m) to the surface, resulting in salt accumulation on, or close to the soil surface, thus these areas are not cultivated. In comparison with the Solonetz soils, where the depth of the sodium ( $\text{Na}^+$ ) rich groundwater table is lower (approximately between 1.5-3.0 m from the soil surface), thus the  $\text{Na}^+$  accumulation is usually not presented at the soil surface, but deeper in the soil, forming a “Natric horizon”. However, cultivation of Solonetz could lead to the mixing of Natric horizon (high pH and  $\text{Na}^+$  content) with the above layered slightly acidic horizon(s) (Stefanovits, 1971; Szabolcs, 1966). A study in Australia, according to Hatton et al. (2003) showed that the broadscale clearing of native deep-rooted perennial vegetation, and its replacement with shallow-rooted annual crops and pastures that alter the hydrologic balance and mobilize salts in the landscape, finally lead to salinization and sodification. Before salinization, salt stores generally occur below the major rooting zones of native vegetation and are largely immobile before land clearing. Another explanation is that the higher plant coverage has higher underground biomass, root system extracting higher amount of root acids strong enough to decrease the pH as the sampling was from the upper 15 cm densely grown over with roots (Hinsinger, 2003). As in Indian bare lands there is no (< 10%) plant coverage, during the hot summer periods the rapid evaporation brings the salts to the soil surface (Jalili et al., 2011). The lowest levels of pH and EC in India at KSnA (arable land) can be explained by the tillage practices, which loosens the soil and create macropores thus improving water infiltration rate (Wong et al., 2010).

The mean values of AL- $\text{P}_2\text{O}_5$  (671.71  $\text{mg kg}^{-1}$ ), AL- $\text{K}_2\text{O}$  (383.42  $\text{mg kg}^{-1}$ ), av $\text{Mg}^{2+}$  (44.07  $\text{mg kg}^{-1}$ ), av $\text{Ca}^{2+}$  (1075.63  $\text{mg kg}^{-1}$ ) were significantly the highest in case of NSnA site, while av $\text{Na}^+$  (907.62  $\text{mg kg}^{-1}$ ) and moisture content (27.61 %) were the highest at AScP. In India the mean values of AL- $\text{P}_2\text{O}_5$  (7.02  $\text{mg kg}^{-1}$ ), AL- $\text{K}_2\text{O}$  (119.04  $\text{mg kg}^{-1}$ ), av $\text{Mg}^{2+}$  (2910  $\text{mg kg}^{-1}$ ), av $\text{Ca}^{2+}$  (5248.5  $\text{mg kg}^{-1}$ ) and moisture (7.18 %) were significantly higher in case of KSnP site. The

different nutrient contents of the soils (AL-P<sub>2</sub>O<sub>5</sub>, AL-K<sub>2</sub>O) can be explained by the applied nutrient management practices and salinity or sodicity. The soil pH affects the forms, bioavailability and dynamics of the nutrient uptake in the soil, i.e., the bioavailability of P is strongly related to soil pH, the pH between 5.5 and 7 constitutes the optimum range for P release (Muhammad et al., 2007; Sanyal and De Datta, 1991).

In our study, in accordance with Grattan and Grieve (1998) and Fageria et al. (2011) Na<sup>+</sup> and salinity competitively inhibit the uptake of plant available nutrients in salt-affected soils. However, avCa<sup>2+</sup>, avMg<sup>2+</sup> and avNa<sup>+</sup> were higher in Indian salt-affected soils presumably due to natural properties of soils or could be due to the higher groundwater movement associated with evaporation and increasing ion concentrations at soil surface (Jalili et al., 2011). Overall, OC, AL-P<sub>2</sub>O<sub>5</sub>, AL-K<sub>2</sub>O and moisture content were higher at Hungarian sites, while, pH, avMg<sup>2+</sup>, avCa<sup>2+</sup> and avNa<sup>+</sup> were higher in case of Indian sites (Table 11).

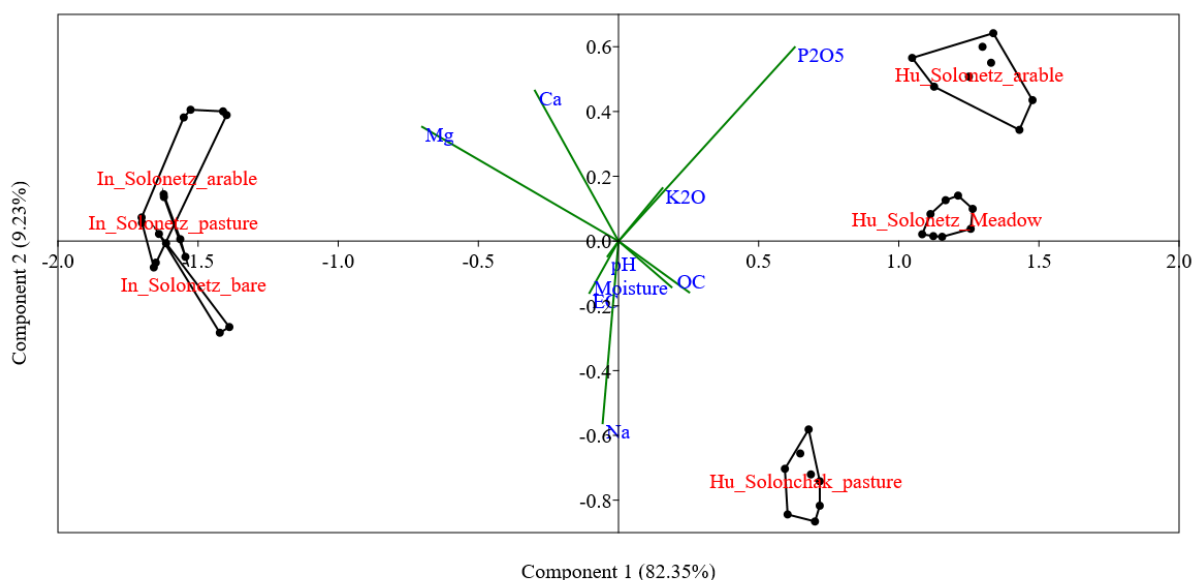
Beside comparing the individual parameters separately using One-way ANOVA, we also investigated whether there are significant differences among the sites, thus One-way ANOSIM analysis was performed based on chemical properties and moisture content (p values can be seen in Table 12). Based on the investigated chemical parameters and soil moisture content, the six sites are statistically different from each other.

**Table 12:** Result of one-way ANOSIM analysis based on chemical properties and moisture content from two sampling seasons of year 2016 samples (R= 0.9669, p=0.0001)

	AScP	NSnA	NSnM	KSnB	KSnP
NSnA	0.0003				
NSnM	0.0003	0.0003			
KSnB	0.0016	0.0014	0.0014		
KSnP	0.0022	0.0015	0.0022	0.0266	
KSnA	0.0002	0.0002	0.0003	0.0018	0.0025

The result of the principal component analysis (PCA) of Hungarian and Indian soils (Figure 5) showed the soil chemical properties and moisture content influenced the grouping of the sample plots, which confirmed the results of the One-way ANOVA (Table 11) and One-way ANOSIM analysis (Table 12). 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<sub>2</sub>O<sub>5</sub> and negatively by avMg<sup>2+</sup> which explains more than 82% of the variance. While the second component explains more than 9% of the variance and determined positively by AL-P<sub>2</sub>O<sub>5</sub>, avCa<sup>2+</sup> and negatively by avNa<sup>+</sup>. Thus, AL-P<sub>2</sub>O<sub>5</sub>, avMg<sup>2+</sup>, avNa<sup>+</sup> and avCa<sup>2+</sup> content of the soils were the main

factors and other parameters had an influence on the site characterization. The Hungarian and Indian locations were clearly separated from each other based on the component 1, (AL-P<sub>2</sub>O<sub>5</sub> and avMg<sup>+</sup> content), as overall the Hungarian sites contain more AL-P<sub>2</sub>O<sub>5</sub> and less avMg<sup>+</sup>. The plots within Hungarian sites were well distinguished primarily by the AL-P<sub>2</sub>O<sub>5</sub>, avNa<sup>+</sup> and avCa<sup>2+</sup> content (Figure 5) due to the soil references groups and land use management practices (NSnA, NSnM, ASnP), as the arable Solonetz samples contain more AL-P<sub>2</sub>O<sub>5</sub> and avCa<sup>2+</sup> and less avNa<sup>+</sup> than the Solonetz pasture and Solonchak pasture ones. The Indian pasture sites (KSnP) and bare lands (KSnB) are overlapping with the arable sites (KSnA). Arable Indian sites (KSnA) are representing the lowest Na<sup>+</sup> and Al- P<sub>2</sub>O<sub>5</sub> content, thus can be separated from the bare sites (KSnB) containing the highest avNa<sup>+</sup> amount, and the pasture sites (KSnP) placed between the arable and bare lands as having the highest Al- P<sub>2</sub>O<sub>5</sub> and avCa<sup>2+</sup> contents.



**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).

#### 4.1.2 Effect of land use on soil microbiological properties

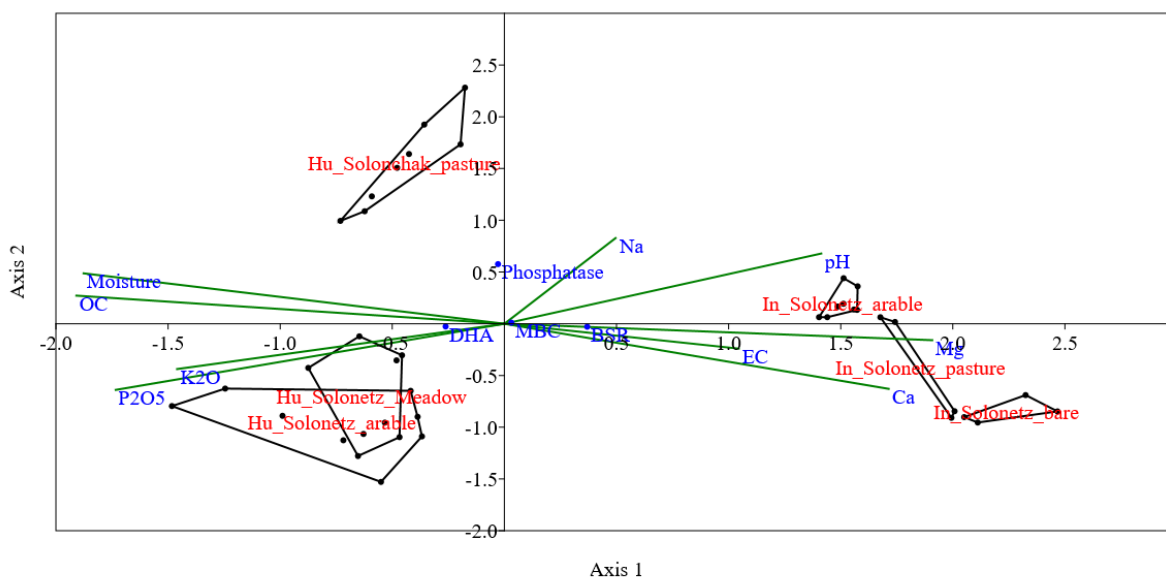
The measured soil microbiological parameters are regularly used indicators for investigating soil health and fertility (Kennedy and Papendick, 1995; Pankhurst et al., 1995; Nielsen and Winding, 2002; Alhameid et al., 2019), revealing high differences between Hungarian and Indian salt-affected soils.

Soil microbiological parameters showed lower values in case of Indian sites (Table 13), except for the BSR (ranging from 6.92 to 10.74  $\mu\text{g CO}_2 \text{ g}^{-1} \text{ soil hr}^{-1}$ ), presumably due to the higher salt concentrations, which could reduce the activity of soil microbes (Batra and Manna, 1997; Rietz

and Haynes, 2003). Weldmichael et al. (2020a) reported that there is a strong positive correlation between soil organic carbon content and BSR values while in our case, the BSR values of investigated sites of both countries showed similar values, but the SOC were significantly different from each other. 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). Silveira et al. (2009) reported that soil physical, chemical and biological properties have strong relationship with organic matter content while according to Tejada et al. (2006) the increasing OC content resulted in an increasing microbial enzyme activity. The positive effect of applied OM on bacterial community was reported by Rousk et al. (2011), adding 2% of organic matter to salt-affected soils resulted in 20-40% increase of bacterial community within a one-month incubation period while 40-60% decrease was found without OM application, indicating that OM has stronger effect on soil microbial community than the salt content of the soil. Moreover, the soil moisture content was about 3-times higher in Hungarian soils resulting in more favorable environmental conditions for microbial life (Iovieno and Bååth, 2008).

**Table 13:** Descriptive statistics (mean and standard deviation) of soil microbiological properties and results of the Tukey's test of the six sampling sites from two sampling seasons of year 2016 samples.

Property	Unit	Hungary			India		
		AScP (n=8)	NSnA (n=8)	NSnM (n=8)	KSnb (n=4)	KSnP (n=4)	KSNA (n=8)
		Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
<b>BSR</b>	( $\mu\text{g CO}_2$ $\text{g}^{-1}$ soil $\text{hr}^{-1}$ )	10.79 (3.14)Bb	5.12 (2.16)Aa	9.71 (2.48)Bb	6.92 (0.61)Aa	9.30 (0.94)Bb	10.74 (0.78)Bc
<b>MBC</b>	( $\mu\text{g C g}^{-1}$ soil)	542.11 (292.79)B b	73.74 (1.14)Aa	575.64 (180.64)Bb	7.23 (3.23)Aa	12.56 (2.75)Ab	46.76 (4.11)Ac
<b>DHA</b>	( $\mu\text{g}$ formazan $\text{g}^{-1}$ soil $\text{day}^{-1}$ )	263.19 (131.82)C b	103.26 (31.92)Ba	332.76 (109.11)Cc	1.43 (0.40)Aa	2.56 (0.58)Ab	3.52 (0.46)Ac
<b>Phosp.</b>	( $\mu\text{mol}$ PNP $\text{g}^{-1}$ soil $\text{hr}^{-1}$ )	0.67 (0.09)Dc	0.11 (0.03)ABCa	0.16 (0.08)Cb	0.06 (0.01)Aa	0.10 (0.05)ABc	0.15 (0.02)BCb
<p><i>a-c small letters indicate significant differences of means within locations between sites according to the Tukey's test (<math>p &lt; 0.05</math>)</i></p> <p><i>A-D capital letters indicate significant differences of means within sites of both countries according to the Tukey's test (<math>p &lt; 0.05</math>)</i></p> <p>where, BSR- basal soil respiration; MBC-microbial biomass carbon; DHA- dehydrogenase activity; Phosp.- alkaline phosphatase activity</p> <p>(Hungary: AScP = Solonchak pasture land; NSnA = Solonetz arable land; NSnM = Solonetz pasture land India: KSNA = Solonetz arable land; KSnb = Solonetz bare land; KSnP = Solonetz pasture land)</p>							



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

The BSR measured in the two countries were similar in range (Table 13) however, the MBC were higher in the Hungarian sites (ranging from  $73.74 \mu\text{g C g}^{-1}$  soil to  $575.64 \mu\text{g C g}^{-1}$  soil) than in India (ranging from  $7.23 \mu\text{g C g}^{-1}$  soil to  $46.76 \mu\text{g C g}^{-1}$  soil). Osmotic stress could result in physiologically more active microbes that used substrate less efficiently (Wichern et al., 2006). Moreover, increased soil respiration (BSR) from Indian arable lands ( $10.74 \mu\text{g CO}_2 \text{ g}^{-1} \text{ soil hr}^{-1}$ ) could be due to the soil disturbance (ploughing) which is also supported by Dalal et al. (2005), who stated that clearing vegetation for pasture or cropping system deteriorates the soil health and results in increased greenhouse gas emissions as most of the SOC concentrated on 0-5 cm depth, conversion of such lands to arable lands may result in the release of a large amount of  $\text{CO}_2$  from the soil surface (Gelaw et al., 2014).

In salt-affected soils, MBC can serve as sensitive indicator of changes in soil organic matter (Yuan et al., 2007) and microbial activities (Wick et al., 1998), and relevantly influenced by the land use practices (Chaudhary et al., 2018). Changes in land use systems from pasture to arable increase the aeration of the soil therefore decreasing the organic matter content. Soil organic matter and the MBC usually are in strong positive correlation and the decreasing organic matter content has strong effect on climate change, too, through the increased  $\text{CO}_2$  emission from soils (Gelaw et al., 2014). Franzluebbers et al. (1995) proved that the soil respiration is influenced by the altering OM content under different management practices.

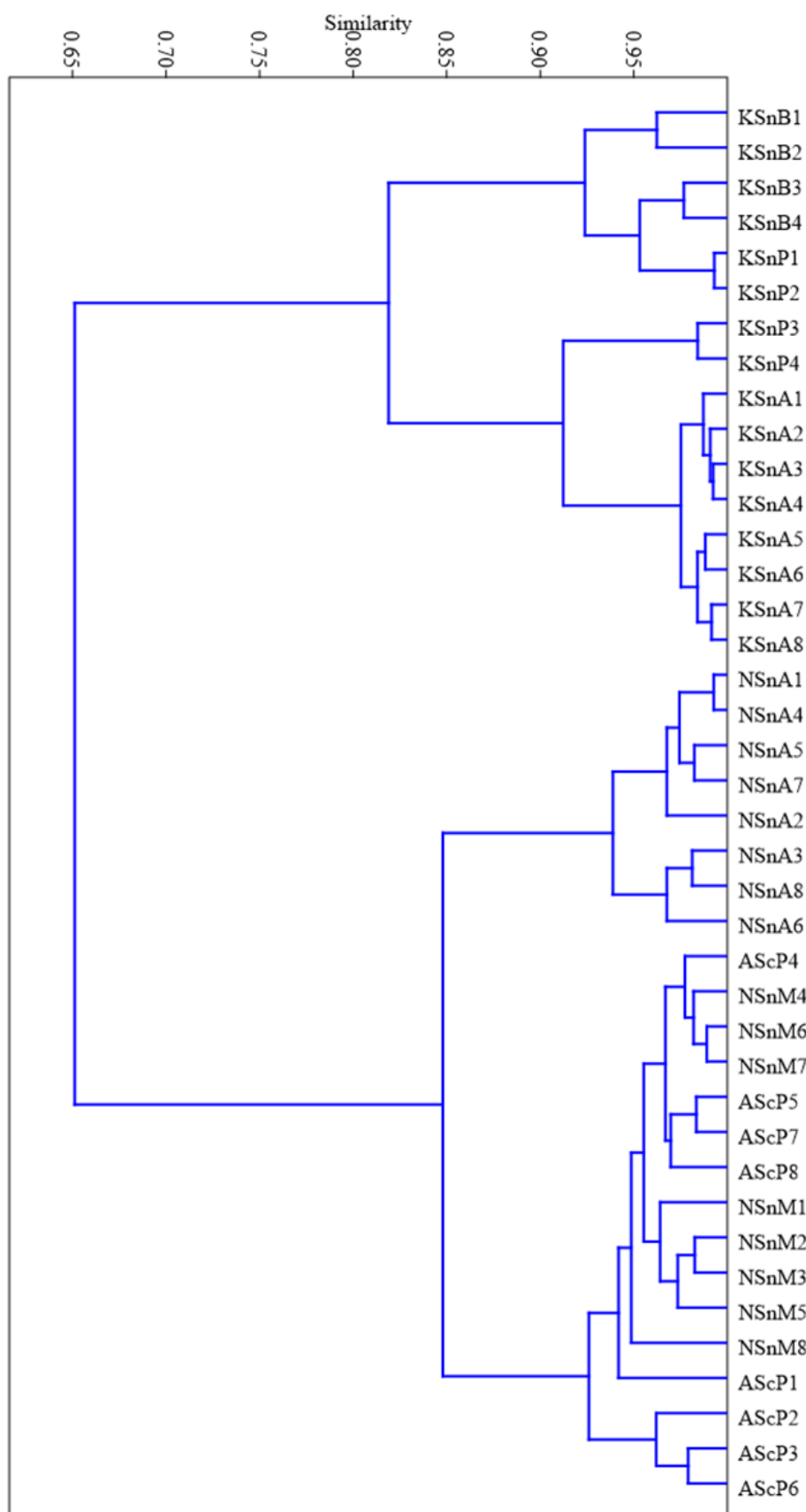
KSnB site in India showed the lowest MBC values ( $7.23 \mu\text{g C g}^{-1}$  soil) with the highest  $\text{Na}^+$  values ( $947 \text{ mg kg}^{-1}$ ) (Table 11 & 13). This result supports the findings of Iwai et al., (2012) who reported

a negative correlation (-0.91) between MBC and  $\text{Na}^+$ . Also, the lower organic carbon content at Indian sites may not provide adequate substrate for microbial activity which is also reported by Sparling (1997) who stated that higher MBC in soils were due to higher organic carbon content. MBC and dehydrogenase activity (DHA) were the highest in Hungarian pasture (NSnM).

According to Rietz and Haynes (2003) alkaline phosphatase activity tends to decrease with increasing salinity and sodicity (SAR). Cultivated land tends to have higher alkaline phosphatase activity compared to uncultivated soil (Zhang et al., 2014) which supports our findings in case of Indian soils. Lemanowicz and Bartkowiak (2016) reported higher alkaline phosphatase activity in the soil deficient in soil phosphorus but the results of Hungarian soils proved that higher alkaline phosphatase activities were observed with higher soil phosphorus contents.

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. Similarly, to the PCA analyses (Figure 5) the Indian arable (KSnA) and bare (KSnB) lands form two different clusters, where pasture (KSnP) sites represent transitional places between the arable and bare lands, two sites of pasture land belong to arable sites, while the other sites belong to the bare land cluster.

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,  $\text{K}_2\text{O}$  and  $\text{P}_2\text{O}_5$  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).



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

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

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

Land use types may have positive and/or negative effects on soil physical, chemical and biological properties (Steenwerth et al., 2002; Bossio et al., 2005; Xu et al., 2017). Organic matter input, preferable soil pH, neutral or slightly alkaline, and accumulation of nutrients improved the soil biological status (Kooch et al. 2018; Negasa 2020). Furthermore, soil microbial properties and enzyme activities were influenced by soil organic matter content affected by the land use or management practices (Meena and Rao, 2021).

Chernozems are the most fertile soils in Hungary, covered with ancient grassy vegetation, on loessy soil parent material with dominant biological processes and high soil organic matter content providing perfect medium for successful plant production. As a result, these soils are under cultivation for hundreds of years (Szűcs 1959).

The soil organic carbon (OC) was found the lowest in Szappanszék-Gleysols-SGIP1 (0.84 %) and the highest in Nádudvar- Chernozems-NChA2 (7.82%) (Table 14). The low soil OC content and variability in case of Szappanszék Gleysols could be due to the soil forming factors as that area was a salt-affected lake, and because of the global climate change the groundwater table has decreased, and as a consequence the area turned to grassland (Wiesmeier et al., 2014; Tóth et al., 2015). In case of SGIP location, statistical difference was observed within the sampling sites. Both Nádudvar- Solonetz soils (NSnA and NSnM) were not significantly different in terms of OC. The Apaj sites (AScP) represented statistically higher values than the Solonetz locations (NSnA and NSnM). The relatively high organic carbon content in case of Solonchak was due to the continuous plant coverage and the rare disturbance (Tejada et al. 2006; Ayoubi et al., 2020). The 15 years of cultivation decreased the organic matter content of the ploughed layer by 12-22% of Solonetz pasture in Hungary (Ábrahám and Ginál, 1967). Land use changes from native vegetation to cropping and continuous cropping decreases the organic carbon content of soils (Guo and Gifford, 2002).

The E4/E6 ratio was ranged from 3.90 to 6.77. The E4/E6 values at SGIP1 and NChA2, and SGIP2 and NSnM1 were not significantly different. The E4/E6 ratio was the highest at SGIP1 (Table 14) suggesting the lower quality of organic matter as results of the vegetation and humus transformation driven by soil microbes. The decomposition of humus could be due to the interaction between stable humus and soil microorganisms resulting in alteration of soil organic matter, which indicates a close correlation between microorganisms and humus formation (Dou



and Wang, 2011). However, at NSnA, NSnM and AScP the E4/E6 values were lower than 5 indicating that the area was characterized by humic acids (Stevenson, 1994).

Soil pH was significantly different at NChA and SGIP sites with the highest observed value at SGIP2 (9.57) while it was the lowest at NChA2 (6.13) whereas the EC was ranged from 48.10  $\mu\text{S cm}^{-1}$  (NChA2) to 392.87  $\mu\text{S cm}^{-1}$  (AScP1) (Table 14). Soil pH values varied from neutral to alkaline (ranging from 6.97 to 9.57) except NChA which was slightly acidic. The significant differences in the EC values and pH of all the sites can be explained by the different soil types and land use / management practices and intensity of agriculture (Assefa et al., 2020). However, EC values at one of the Gleysol pasture sites (SGIP) was lower which could be due to the drainage processes (Tóth et al., 2015; Molnár et al., 2019).

The values of  $\text{P}_2\text{O}_5$ ,  $\text{K}_2\text{O}$ ,  $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$  were found higher at both arable sites (NSnA and NChA) compared to meadow (NSnM) and pasture sites (AScP and SGIP) (Table 14). At NSnA and NSnM the values of  $\text{K}_2\text{O}$  were significantly different whereas at NChA and NSnM,  $\text{P}_2\text{O}_5$  were significantly different.  $\text{Na}^+$  was found the highest at AScP1 (789.00  $\text{mg kg}^{-1}$ ) and the lowest at SGIP1 (172.33  $\text{mg kg}^{-1}$ ) which is not significantly different from NChA. In case of soil moisture, AScP1 had the highest value (32.28  $\text{mg kg}^{-1}$ ) and SGIP1 had the lowest value (15.42  $\text{mg kg}^{-1}$ ). Soil moisture was found statistically different in all sites except NSnA2 and AScP1. Explaining the high salt, sodium content and soil moisture of salt-affected soils, the differences in  $\text{Na}^+$  content and soil moisture in the sampling sites were the same as it was explained earlier in the subchapter 4.1.1.

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  $\text{P}_2\text{O}_5$  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  $\text{P}_2\text{O}_5$  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.

**Table 14:** Descriptive statistics ANOVA (mean  $\pm$  standard deviation (SD)) of the soil moisture and chemical properties from year 2017 samples.

Property	Unit	NSnA		NChA		NSnM		AScP		SGIP	
		NSnA1	NSnA2	NChA1	NChA2	NSnM1	NSnM2	AScP1	AScP2	SGIP1	SGIP2
		Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD
OC	(%)	3.48 $\pm$ 0.12c	3.52 $\pm$ 0.20c	7.51 $\pm$ 0.26e	7.82 $\pm$ 0.04e	3.37 $\pm$ 0.06c	3.56 $\pm$ 0.05c	6.21 $\pm$ 0.81d	5.81 $\pm$ 0.19d	0.84 $\pm$ 0.05a	1.77 $\pm$ 0.08b
E4/E6		4.17 $\pm$ 0.06b	4.23 $\pm$ 0.06b	5.17 $\pm$ 0.06d	6.70 $\pm$ 0.20e	3.90 $\pm$ 0.00a	4.80 $\pm$ 0.00c	4.77 $\pm$ 0.06c	4.77 $\pm$ 0.06c	6.77 $\pm$ 0.06e	3.90 $\pm$ 0.00a
pH		6.97 $\pm$ 0.06c	7.00 $\pm$ 0.10c	6.77 $\pm$ 0.06b	6.13 $\pm$ 0.06a	8.47 $\pm$ 0.06e	8.47 $\pm$ 0.06e	8.10 $\pm$ 0.00d	8.10 $\pm$ 0.00d	9.00 $\pm$ 0.10f	9.57 $\pm$ 0.06g
EC	( $\mu$ S cm <sup>-1</sup> )	136.37 $\pm$ 0.97bc	108.33 $\pm$ 6.21b	156.87 $\pm$ 11.29c	48.10 $\pm$ 1.91a	144.77 $\pm$ 6.12c	131.17 $\pm$ 4.05bc	392.87 $\pm$ 16.77e	262.20 $\pm$ 20.42d	51.20 $\pm$ 3.21a	141.73 $\pm$ 12.71c
P2O5	(mg kg <sup>-1</sup> )	420.33 $\pm$ 18.15d	410.00 $\pm$ 13.89d	545.00 $\pm$ 39.69e	650.00 $\pm$ 6.24f	283.67 $\pm$ 19.35c	162.00 $\pm$ 4.58b	55.80 $\pm$ 3.10a	48.77 $\pm$ 4.86a	31.50 $\pm$ 1.71a	42.90 $\pm$ 4.25a
K2O	(mg kg <sup>-1</sup> )	377.67 $\pm$ 20.82d	471.67 $\pm$ 24.01e	303.67 $\pm$ 11.02c	298.33 $\pm$ 12.86c	352.00 $\pm$ 19.29d	187.67 $\pm$ 9.87b	203.67 $\pm$ 15.95b	196.00 $\pm$ 19.08b	43.43 $\pm$ 2.74a	83.43 $\pm$ 4.69a
Mg	(mg kg <sup>-1</sup> )	44.23 $\pm$ 10.66bc	42.33 $\pm$ 12.88abc	34.20 $\pm$ 9.65abc	37.20 $\pm$ 7.85abc	32.27 $\pm$ 8.69abc	31.43 $\pm$ 6.40abc	42.23 $\pm$ 8.42abc	49.57 $\pm$ 9.77c	18.90 $\pm$ 4.17a	19.53 $\pm$ 3.80ab
Ca	(mg kg <sup>-1</sup> )	1495.00 $\pm$ 385.52bc	1411.67 $\pm$ 297.36bc	1843.00 $\pm$ 434.25c	1859.33 $\pm$ 515.51c	899.00 $\pm$ 192.01ab	834.67 $\pm$ 260.34ab	668.33 $\pm$ 162.11ab	738.33 $\pm$ 187.50ab	145.67 $\pm$ 44.38a	116.83 $\pm$ 33.25a
Na	(mg kg <sup>-1</sup> )	350.00 $\pm$ 82.83ab	271.33 $\pm$ 45.83ab	237.67 $\pm$ 39.07a	227.00 $\pm$ 41.58a	498.00 $\pm$ 94.18bc	335.33 $\pm$ 78.87ab	789.00 $\pm$ 138.01d	692.00 $\pm$ 141.45cd	172.33 $\pm$ 45.00a	357.33 $\pm$ 64.69ab
Moisture	(%)	26.93 $\pm$ 2.90bc	31.27 $\pm$ 6.82c	18.71 $\pm$ 1.18ab	23.74 $\pm$ 1.70abc	23.85 $\pm$ 5.61abc	16.79 $\pm$ 1.83ab	32.28 $\pm$ 2.23c	22.27 $\pm$ 4.46abc	15.42 $\pm$ 3.72a	18.01 $\pm$ 3.78ab
<i>a-g letters indicate significant differences of means according to the Tukey's test (<math>p &lt; 5</math>)</i> <i>Site abbreviations are used according to Table 2.</i>											



heterogeneity in terms of microbiological activity in the area which could be attributed to the greater root mass on permanent grassy vegetation. The measured soil microbiological parameters are regularly used indicators for investigating soil health and fertility (Kennedy and Papendick, 1995; Pankhurst et al., 1995; Nielsen and Winding, 2002; Alhameid et al 2019), revealing high differences between different management practices. The lower microbial activities in both arable lands (NSnA and NChA) could be the result of ploughing which disturbed and homogenised the soil and decreased the soil microbial activity, while the continuous plant coverage resulted in an undisturbed environment, and an increase in microbial enzyme activities in pasture and meadow sites (Tejada et al., 2006).

#### **4.2.2 Effects of land use and soil types on PLFA composition**

The variation of microbial activity amongst the different land use practices is probably associated with the soil moisture which played an important role in the diversification of microbial activities (CCA, Figure 10). Weldmichael et al. (2020b; 2021), reported positive influence of soil moisture on BSR of different soil types in Hungary. Also, the role of soil water availability and salinity in soil microbial community composition is relevant in forest systems and coastal soils, respectively (Drenovsky et al., 2010; Yan et al., 2021). When considering each microbial parameter individually, some significant differences were found within land use practices. Soil microbial parameters were able to distinguish abandoned area from extensive cropping and intensive pasture land (Costa et al., 2013), whereas others (Qi et al., 2018; Zhu et al., 2021) observed significant changes in soil physical, chemical properties and microbial biomass after land use changes. Furthermore, Tilston et al. (2010) stated that soil microbial community usually strongly changed in response to the current land use practices.

The results of this study give new insights into the relationships of soil chemical and microbial properties of salt-affected soils under different land use practices because PLFAs represent in situ microbial community composition and biomass size of soils (Kaur et al., 2005). Cultivating Chernozem soils as an arable land could decrease the size of its microbial community to the third of the microbial community size of the salt affected Solonetz meadow and Solonchak pasture lands.

The highest value of general bacterial PLFAs was measured in NSnM2 site (14.6408 nmol g<sup>-1</sup> soil) while this value was somehow lower at AScP1 (13.4842 nmol g<sup>-1</sup> soil) and AScP2 (14.0466 nmol g<sup>-1</sup> soil) sites. General bacterial PLFA values indicated smaller bacterial community at sites NSnM1 (10.8295 nmol g<sup>-1</sup> soil) and SGIP2 (7.2052 nmol g<sup>-1</sup> soil), followed by the arable sites (NSnA1, NSnA2, NChA1, NChA2) and site SGIP2 in a range of 4.0117-5.5699 nmol g<sup>-1</sup> dry soil (Table 15).

**Table 15:** Descriptive statistics ANOVA (mean  $\pm$  standard deviation (SD)) of the classical soil microbiological properties from year 2017 samples

		NSnA		NChA		NSnM		AScP		SGIP	
		NSnA1	NSnA2	NChA1	NChA2	NSnM1	NSnM2	AScP1	AScP2	SGIP1	SGIP2
Property	Unit	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean SD	Mean $\pm$ SD	Mean $\pm$ SD
BSR	( $\mu\text{g CO}_2 \text{ g}^{-1}$ soil $\text{hr}^{-1}$ )	1.59 $\pm$ 0.30a	5.13 $\pm$ 0.88d	2.78 $\pm$ 0.23bc	2.78 $\pm$ 0.23bc	5.38 $\pm$ 0.05d	2.03 $\pm$ 0.08ab	5.42 $\pm$ 0.23d	3.09 $\pm$ 0.12c	2.96 $\pm$ 0.14bc	3.07 $\pm$ 0.14c
MBC	( $\mu\text{g C g}^{-1}$ )	197.51 $\pm$ 26.59b	182.09 $\pm$ 27.10ab	74.86 $\pm$ 0.04a	182.34 $\pm$ 26.57ab	493.14 $\pm$ 74.20d	414.36 $\pm$ 5.63cd	735.80 $\pm$ 24.41e	717.18 $\pm$ 58.05e	159.03 $\pm$ 24.35ab	320.25 $\pm$ 47.37c
DHA	( $\mu\text{g}$ formazan $\text{g}^{-1}$ soil $\text{day}^{-1}$ )	39.42 $\pm$ 0.09b	82.36 $\pm$ 0.26d	83.47 $\pm$ 0.98d	76.37 $\pm$ 0.21c	282.40 $\pm$ 0.67g	152.14 $\pm$ 1.23f	520.64 $\pm$ 1.69i	378.99 $\pm$ 0.51h	4.95 $\pm$ 0.03a	102.89 $\pm$ 1.03e
Phosphatase	( $\mu\text{mol PNP}$ $\text{g}^{-1}$ soil $\text{hr}^{-1}$ )	0.09 $\pm$ 0.000a	0.09 $\pm$ 0.000a	0.13 $\pm$ 0.001d	0.12 $\pm$ 0.001c	0.27 $\pm$ 0.001f	0.24 $\pm$ 0.001e	0.83 $\pm$ 0.006h	0.73 $\pm$ 0.002g	0.13 $\pm$ 0.000d	0.10 $\pm$ 0.001b
<p><i>a-i letters indicate significant differences of means according to the Tukey's test (<math>p &lt; 5</math>)</i></p> <p><i>Site abbreviations are used according to Table 2.</i></p>											

In case of Gram-positive bacterial PLFAs the highest value was measured also at NSnM2 site (16.6953 nmol g<sup>-1</sup> soil) followed by AScP2 (16.0713 nmol g<sup>-1</sup> soil), AScP1 (16.2817 nmol g<sup>-1</sup> soil) and NSnM1 (13.6949 nmol g<sup>-1</sup> soil) sites. As intermediate values, 7.4337 nmol g<sup>-1</sup> dry soil PLFAs was measured at SGIP2 site, followed by 6.1866 and 5.3238 nmol g<sup>-1</sup> dry soil PLFAs measured at NSnA2 and NSnA1 sites, respectively. The lowest values were found at NChA2 (4.8926 nmol g<sup>-1</sup> soil) and NChA1 (5.0884 nmol g<sup>-1</sup> soil) sites.

The range of measured Gram-negative bacterial PLFAs revealed lower Gram-negative bacterial community in soils than Gram-positive ones. Results indicated the highest Gram-negative bacterial community at AScP2 (4.4483 nmol g<sup>-1</sup> soil) and AScP1 (4.3579 nmol g<sup>-1</sup> soil) sites. The intermediate results were measured at NSnM2 (3.9085 nmol g<sup>-1</sup> soil) and NSnM1 (3.0585 nmol g<sup>-1</sup> soil) sites whose mean values were only the 79.09% of AScP sites, compared to the 93.93% measured in case of Gram-positive PLFA indicators. SGIP2 site (2.6449 nmol g<sup>-1</sup> soil) also had higher PLFA content than arable and SGIP1 sites (1.2718-1.6834 nmol g<sup>-1</sup> dry soil).

The highest Actinobacteria community was found in AScP1 (9.0212 nmol g<sup>-1</sup> soil) and AScP2 (7.7977 nmol g<sup>-1</sup> soil) sites while the corresponding PLFA concentrations were much lower at NSnM2 (4.4095 nmol g<sup>-1</sup> soil) and NSnM1 (3.4946 nmol g<sup>-1</sup> soil) sites followed by the arable and Gleysol pasture sites (2.2540-1.3459 nmol g<sup>-1</sup> soil).

The volume of AMF community was more similar in case of AScP and NSnM sites compared to Actinobacteria results. In detail, the highest values were measured at NSnM2 (0.6482 nmol g<sup>-1</sup> soil) and NSnM1 (0.5909 nmol g<sup>-1</sup> soil) sites followed by the results of NSnM2 (0.4942 nmol g<sup>-1</sup> soil) and NSnM1 (0.4024 nmol g<sup>-1</sup> soil) sites. At the remaining arable and Gleysol pasture sites the PLFA indicated smaller AMF communities with the range of 0.1453-0.1767 nmol g<sup>-1</sup> dry soil values.

Fungal communities were higher at AScP2 (2.0899 nmol g<sup>-1</sup> soil), NSnM2 (1.5070 nmol g<sup>-1</sup> soil), NSnM1 (1.4777 nmol g<sup>-1</sup> soil), AScP1 (1.4207 nmol g<sup>-1</sup> soil) and SGIP2 (1.1597 nmol g<sup>-1</sup> soil) sites than at SGIP1 (0.6027 nmol g<sup>-1</sup> soil), NSnA1 (0.5067 nmol g<sup>-1</sup> soil), NChA2 (0.3980 nmol g<sup>-1</sup> soil), NSnA2 (0.3613 nmol g<sup>-1</sup> soil) and NChA1 (0.2505 nmol g<sup>-1</sup> soil) sites.

Summarizing the results of different microbial groups, the largest communities were found at AScP1 (45.1566 nmol g<sup>-1</sup> soil) and AScP2 (45.1022 nmol g<sup>-1</sup> soil) sites followed by NSnM2 (41.6553 nmol g<sup>-1</sup> soil) and NSnM1 (32.9575 nmol g<sup>-1</sup> soil) sites. While the total PLFA results were similar in case of AScP sites, the two sites of NSnM land were different from each other. Highest difference in PLFA communities was found in SGIP sites where 21.1990 nmol g<sup>-1</sup> dry soil PLFA concentration was measured in SGIP2 site while this value was only 13.6606 nmol g<sup>-1</sup> soil in SGIP1 site. In the arable sites the total PLFA content was much lower than in AScP and NSnM

sites, ranging from 13.3344 nmol g<sup>-1</sup> soil to 14.8196 nmol g<sup>-1</sup> soil with higher values of NSnA sites than NChA sites.

Ratios of PLFA groups indicate the biological properties of soils in different sites as a function of environmental circumstances (Table 17). The lowest ratios of G-negative/G-positive bacteria were found at NSnM1 (0.2233), NSnM2 (0.2341) and NSnA2 (0.2056) sites followed by AScP1 (0.2677), SGIP1 (0.2702) and AScP2 (0.2768) sites. Higher results were measured at the arable NSnA1 (0.2947), NChA2 (0.3093) and NChA1 (0.3309), moreover the pasture SGIP2 (0.3558) sites.

Means of fungi/general bacterial PLFAs also were separated in different groups. Considerably low ratio was found at the NChA1 (0.0624) site, which was followed by NSnA2 site (0.0765). The third group contained the remained arable sites NChA2 (0.0989), NSnA1 (0.1073), the meadow NSnM2 (0.1029) site and, the pasture AScP1 (0.1054) and SGIP1 (0.1082) sites. Highest ratios were calculated for NSnM1 (0.1365), AScP2 (0.1488) and SGIP2 (0.1610) sites.

The Actinobacteria to general bacteria ratios varied strongly within land use practices. The ratio was low at two studied lands: the lowest ratio was calculated for the SGIP1 (0.2416) site followed by NSnM2 (0.3012), NSnM1 (0.3227) and SGIP2 (0.3442) sites. The Solonetz arable sites (NSnA1 and NSnA2) had similar ratios (0.4114 and 0.4447, respectively). The highest ratios were counted for Solonhaks pasture and Chernozem arable sites from 0.5551 to 0.6690.

Fungi have an important role in good soil structure (Eash et al., 1996). Filamentous fungi are more sensitive to physical disturbances like soil tillage than single celled microbes (Kabir et al., 1999) causing a decrease in fungal PLFA and in fungi to bacteria ratio in our sites. The lower fungi to bacteria ratio values of both arable sites indicate the stronger effect of tillage than soil type on the community of soil fungi. The fungal PLFA quantities had the lowest values in Chernozem soil following the other arable land on Solonetz soil which indicate stronger negative effects on Chernozem than Solonetz soil due to the long-standing tillage on Chernozem soil. Jangid et al. (2011) suggested a lasting impact (more than 50 years) of cultivation history on the soil microbial, mainly bacterial community.

**Table 16:** Descriptive statistics ANOVA (mean  $\pm$  standard deviation (SD)) of the soil PLFA properties (nmol PLFA g<sup>-1</sup> soil) from year 2017 samples

Property	NSnA1	NSnA2	NChA1	NChA2	NSnM1	NSnM2	AScP1	AScP2	SGIP1	SGIP2
General bacteria $\pm$ SD	4.7206 $\pm$ 0.1634 b	4.7247 $\pm$ 0.0748 b	4.0117 $\pm$ 0.0127 a	4.0345 $\pm$ 0.1620 a	10.8295 $\pm$ 0.0924 e	14.6408 $\pm$ 0.0934 h	13.4842 $\pm$ 0.0089 f	14.0466 $\pm$ 0.0330 g	5.5699 $\pm$ 0.0406 c	7.2052 $\pm$ 0.0405 d
Gram-positive $\pm$ SD	5.3280 $\pm$ 0.1864 b	6.1866 $\pm$ 0.1368 c	5.0884 $\pm$ 0.2001 ab	4.8926 $\pm$ 0.1577 a	13.6949 $\pm$ 0.0667 e	16.6953 $\pm$ 0.2095 g	16.2817 $\pm$ 0.0416 f	16.0713 $\pm$ 0.1356 f	4.7217 $\pm$ 0.0681 a	7.4337 $\pm$ 0.0362 d
Gram-negative $\pm$ SD	1.5714 $\pm$ 0.1037 bc	1.2718 $\pm$ 0.1029 a	1.6834 $\pm$ 0.0658 c	1.5108 $\pm$ 0.0744 b	3.0585 $\pm$ 0.1074 e	3.9085 $\pm$ 0.0245 f	4.3579 $\pm$ 0.0543 g	4.4483 $\pm$ 0.0039 g	1.2755 $\pm$ 0.0136 a	2.6449 $\pm$ 0.0174 d
Actino-bacteria $\pm$ SD	1.9416 $\pm$ 0.0456 b	2.1010 $\pm$ 0.0291 bc	2.5240 $\pm$ 0.1025 c	2.3396 $\pm$ 0.1008 bc	3.4946 $\pm$ 0.0508 d	4.4095 $\pm$ 0.0486 e	9.0212 $\pm$ 0.2580 g	7.7977 $\pm$ 0.3726 f	1.3455 $\pm$ 0.0312 a	2.4802 $\pm$ 0.0154 c
AMF $\pm$ SD	0.1767 $\pm$ 0.0115 b	0.1714 $\pm$ 0.0020 b	0.1711 $\pm$ 0.0083 b	0.1588 $\pm$ 0.0082 ab	0.4024 $\pm$ 0.0015 d	0.4942 $\pm$ 0.0134 e	0.5909 $\pm$ 0.0106 f	0.6482 $\pm$ 0.0028 g	0.1453 $\pm$ 0.0036 a	0.2754 $\pm$ 0.0141c
Fungi $\pm$ SD	0.5067 $\pm$ 0.0108 d	0.3613 $\pm$ 0.0017 b	0.2505 $\pm$ 0.0170 a	0.3980 $\pm$ 0.0223 c	1.4777 $\pm$ 0.0031 h	1.5070 $\pm$ 0.0062 h	1.4207 $\pm$ 0.0007 g	2.0899 $\pm$ 0.0054 i	0.6027 $\pm$ 0.0046e	1.1597 $\pm$ 0.0065f
Total PLFA $\pm$ SD	14.2450 $\pm$ 0.5143 bc	14.8169 $\pm$ 0.2738 c	13.7290 $\pm$ 0.4471 ab	13.3344 $\pm$ 0.1486 a	32.9575 $\pm$ 0.1339 e	41.6553 $\pm$ 0.3215 f	45.1566 $\pm$ 0.2761 g	45.1022 $\pm$ 0.4236 g	13.6606 $\pm$ 0.1481 ab	21.1990 $\pm$ 0.0769 d
a–g small letters indicate significant differences of means between sites according to the Tukey's test ( $p < 0.05$ ).										



**Table 17:** Descriptive statistics of ANOVA (mean  $\pm$  standard deviation (SD)) of the ratios of PLFA groups from year 2017 samples

Property	NSnA1	NSnA2	NChA1	NChA2	NSnM1	NSnM2	AScP1	AScP2	SGIP1	SGIP2
Gram-/Gram+ $\pm$ SD	0.2947 $\pm$ 0.0096 bc	0.2056 $\pm$ 0.0173 a	0.3309 $\pm$ 0.0050 de	0.3093 $\pm$ 0.0246 cd	0.2233 $\pm$ 0.0012 a	0.2341 $\pm$ 0.0026 a	0.2677 $\pm$ 0.0038 b	0.2768 $\pm$ 0.0022 b	0.2702 $\pm$ 0.0020 b	0.3558 $\pm$ 0.0034 e
Fungi/bacteria $\pm$ SD	0.1073 $\pm$ 0.0015 c	0.0765 $\pm$ 0.0009 b	0.0624 $\pm$ 0.0039 a	0.0989 $\pm$ 0.0097 c	0.1365 $\pm$ 0.0009 d	0.1029 $\pm$ 0.0003 c	0.1054 $\pm$ 0.0001 c	0.1488 $\pm$ 0.0007 e	0.1082 $\pm$ 0.0005 c	0.1610 $\pm$ 0.0019 f
Actinobacteria/ General bacteria $\pm$ SD	0.4114 $\pm$ 0.0055 d	0.4447 $\pm$ 0.0009 d	0.6292 $\pm$ 0.0204 f	0.5801 $\pm$ 0.0186 e	0.3227 $\pm$ 0.0067 bc	0.3012 $\pm$ 0.0014 b	0.6690 $\pm$ 0.0193 f	0.5551 $\pm$ 0.0261 e	0.2416 $\pm$ 0.0040 a	0.3442 $\pm$ 0.0002 c
<p>a–f small letters indicate significant differences of means between sites according to the Tukey's test (<math>p &lt; 0.05</math>).</p> <p><i>Site abbreviations are used according to Table 2.</i></p>										

Similarly, the G-negative to G-positive ratio (Table 17) also indicated that degradation processes have taken place in arable lands with higher ratios in arable than pasture and meadow lands. G-negative bacteria have cyclo fatty acids in their cell membrane which can help them to survive in a stressed environmental condition (Guckert et al., 1986). Moreover, G-negative bacteria rapidly assimilate the grasses' rhizodeposit (Treonis et al., 2004) which could explain the highest G-negative bacterial PLFA concentrations in AScP and NSnM sites. Low G-negative PLFA concentration in SGIP sites indicated the strong impact of unfavourable soil chemical properties on microbial community size.

Community size of AM fungi is influenced among others by the presence or absence of host plant (Karasawa et al., 2002) and plant available soil P content (Koide, 1991). In our experiment, host plants were grown on each site. The plant available P content in soil were higher at arable sites (NSnA and NChA) with low AM fungi PLFA content while in case of NSnM and AScP sites low plant available P content and high AMF PLFA content were measured. These results corroborate the role of AM fungi in plant phosphorus acquisition (Kobae, 2019), still in case of SGIP sites the lowest soil P and considerably low AMF PLFA contents were measured. Gleysol pasture sites seem to be a transition between pasture/meadow and arable sites and the soil of this sampling site was very heterogeneous concerning soil microbial properties, mainly PLFA content (Table 15). Moche et al. (2015) also found low concentrations of G-positive bacteria, Actinobacteria and fungi PLFA markers in Eutric Gleysol in Germany, where they found that soil organic carbon and soil texture had the main effect on microbial community. This observation was confirmed by our results with the lowest organic matter content in Gleysol sampling plots.

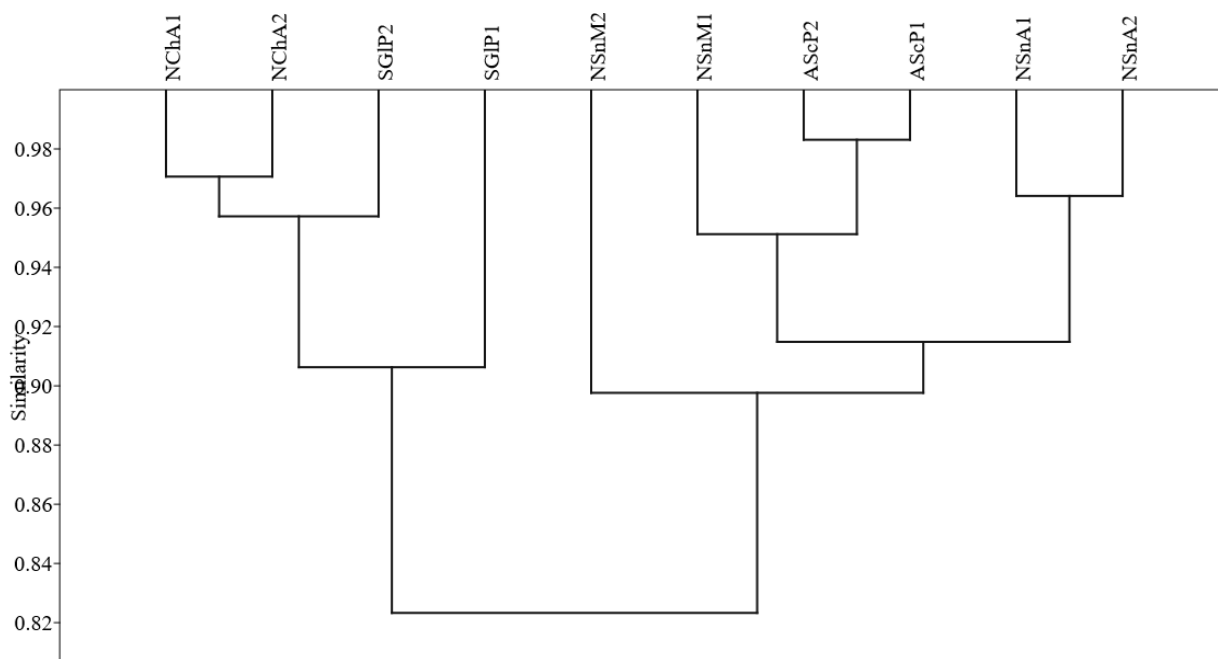
Concerning total PLFA concentrations of studied plots, the undisturbed pasture and meadow soils had higher values than arable sites. Regular tillage of arable sites usually decreased the microbial biomass and richness of arable lands (Zornoza et al., 2009).

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: salt-affected and slightly-salt-affected soils (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<sup>+</sup> content.

One sampling site of Gleysol pasture (SGIP1) was separated to the rest of the cluster which could indicate the high differences in microbiological properties with the other measured sampling sites. This indicates the greatly heterogeneous microbial properties within the sampling site as well.

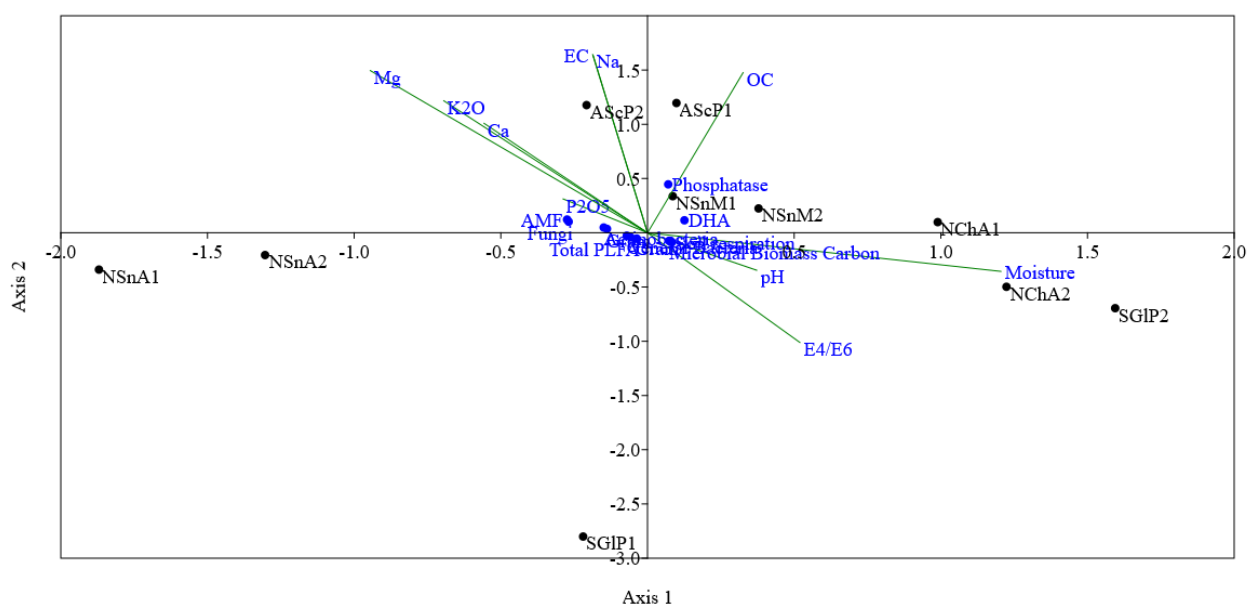
In case of arable sites (NChA, NSnA) the inherited soil properties appearing in the soil classification, have greater influence on the soil community structure than land use. Contrary in case of salt affected soils, the land use was the major driving factor to separate the two sites, as Nádudvar Solonetz meadow (NSnM) sites are closer to Apaj Solonchak pasture (AScP) sites in terms of microbiological properties. The similarity among the Chernozems and Gleysol sites originated from the non-salic, or sodic properties.

Cluster analysis (Bray-Curtis) (Figure 9) resulted in two groups of sampling sites: slightly-salt-affected (NChA and SGIP) and salt-affected (NSnA, NSnM and AScP). Concerning the effect of vegetation cover of pasture and meadow sites this result is in accordance with Jangid et al. (2011) and Rajaniemi and Allison (2009) who found that plant community was not the main driver in microbial community pattern. However, land use has strong effect on soil microbial community (Drenovsky et al., 2010; Van Leeuwen et al., 2017), stronger than vegetation and soil properties and the recovery of damaged microbial community of crop soil needs several years, mainly in bulk soil (Jangid et al., 2011). On the other hand, Bezemer et al. (2006) and Lucas-Borja et al. (2012) did not find any relationship between soil microbial community structure and land-use type.



**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 SGIP2 site while AScP sites separated along the Axes 2 together with SGIP1 site. Loadings of NSnM sites were  $P < 0.05$ .



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

Fuchs et al., (2011) investigated the taxonomic distances among Hungarian soil types based on the soil forming processes, concluded that salt-affected soils ('Solonchaks' and 'Solonetz's') formed a well separated cluster from the other soil classification units, and the soil types are very close to each other with short taxonomic distances, due to the characteristic soil forming processes, like salt and sodium accumulation. While in case of 'Chernozems' soils, the most dominant soil forming process was humification, which resulted that these soils did not form a coherent, distinct taxonomic group, but the different soil types are close to other soil taxonomic units, like 'Gleysols'. These taxonomic distances could be observed based on our investigations as the driving factor is the same, like land use, but the soils formed two well separated clusters where

the most dominant factor was soil type not the land use, as these soils are far from each other in chemical and physical properties, and soil forming processes manifesting in soil classification units. However, within one taxonomic soil unit, or as the soils are close to each other, the land use had a more pronounced effect on soil microbiological properties.

#### **4.3 Effects of different land usage on the bacterial community composition**

Bacterial diversity plays a crucial role in soil ecosystem services and is shaped by the environmental quality of the surroundings. Various land-use practices can impact bacterial diversity through habitat disruption, consequently altering soil characteristics (Mhete et al., 2020) leading to the variations in the abundance of dominant phyla depending on land use and soil depth Ujvári et al., (2020). 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%) (Table 18 & 19). 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.

Recently, the distinct habitat preferences of *Acidobacteriia* and *Blastocatellia* in tundra soil has been observed by Ivanova et al. (2020). It was reported that these two classes of the phylum *Acidobacteriota* have opposite habitat preferences, since *Blastocatellia* were primarily abundant in unfixed sands, while *Acidobacteriia* preferred more developed soils with continuous plant cover. However, 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.

In the case of the soil SGIP the sampled plot was under fully developed plant cover, still the most abundant OTU in this sample (OTU2, 7.5% relative abundance; Table 20) was most closely related to members of the genus *Brevitalea* (*B. aridisoli* and *B. deliciosa*), which were isolated from Namibian semiarid savanna soil (Wüst et al., 2016). 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.

The soil AScP had the second highest sand content and had slightly patchy vegetation cover, while soils NSnA and NChA had markedly lower sand content but were used as arable lands. Considering the fact that soils under intensive agricultural management (e.g., ploughing, tilling)

could result in similar (weak) conditions for microbes than high sand content, it may be concluded that high sand content is the main factor, which determines whether *Blastocatellia* or *Acidobacteria* will be dominant in the acidobacterial community. This observation is in agreement with the finding of Xia et al. (2020), according to which soil texture is one of the most important factors in shaping soil bacterial communities.

**Table 18:** Relative abundance of major bacterial phyla in the soil bacterial communities revealed by Illumina paired-end 16S rDNA amplicon sequencing. (All taxa contributing more than 1% abundance were depicted).

Phylum	NSnA	NChA	NSnM	AScP	SGIP
<i>Acidobacteriota</i>	17.23%	20.96%	25.72%	17.10%	19.56%
<i>Actinobacteria</i>	6.52%	9.77%	19.60%	18.68%	13.13%
<i>Bacteroidetes</i>	6.23%	4.56%	1.80%	2.48%	2.76%
<i>Chloroflexota</i>	1.90%	3.28%	10.62%	4.40%	11.42%
<i>Entothaeonellaeota</i>	0.00%	0.00%	0.00%	1.66%	0.54%
<i>Firmicutes (Bacillota)</i>	1.99%	1.48%	1.13%	0.70%	9.35%
<i>Gemmatimonadetes</i>	13.61%	10.28%	2.02%	4.71%	3.96%
<i>Latescibacteria</i>	0.10%	1.98%	0.00%	0.87%	0.19%
<i>Planctomycetes</i>	8.14%	7.32%	3.16%	4.24%	2.44%
<i>Pseudomonadota</i>	37.41%	33.18%	22.93%	37.35%	34.34%
<i>Rokubacteria</i>	0.16%	2.24%	0.00%	2.30%	0.41%
<i>Verrucomicrobia</i>	5.27%	3.52%	11.98%	3.22%	1.00%
Others	1.43%	1.42%	1.04%	2.27%	0.90%

Moreover, in a study by Mencil et al. (2022b), a significant correlation was found between biochemical parameters (enzymatic activity and microbial abundance) and organic matter components. In another study, it was observed that bacterial community structure was affected by environmental factors such as soil organic matter, soil moisture and EC (He et al. 2021). Similar to *Blastocatellia*, *Vicinamibacteria* were marginal in soil NSnM, but abundant in the other soil samples (Table 19). Members of the class *Vicinamibacteria* showed the highest abundance in soils of arable lands (soils NChA and NSnA), and lower abundances in soils SGIP and AScP. This result can be (at least partly) explained by the observations that these acidobacteria typically inhabit grassland soils and are positively correlated with nutrient availability (Naether et al., 2012; Navarrete et al., 2015). Thus, the abundance maximum of these bacteria in the arable soils can be explained by the high amount of nutrients originated from fertilizers used at these plots. 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. Several studies indicated that pH is the most prominent environmental factor that derives *Acidobacteriota* diversity (Lauber et al., 2009; Griffiths et al., 2011; Conradie and Jacobs, 2021). As pH decreases from neutrality a stronger phylogenetic clustering of the *Acidobacteriota* was observed by several authors (Jones et al., 2009; Griffiths et al., 2011; Nacke et al., 2011). However, an opposite tendency was observed in this study.

Members of *Actinobacteriota* are widely distributed in the soil with high sensitivity to acid and low pH (Anandan et al., 2016) and show maximum growth around neutrality but grow best at a pH between 6 and 9 (Hazarika and Thakur, 2020) as observed in this study. The highest abundance of *Actinobacteriota* was found in NSnM (19.6%) and AScP (18.68%) with a highest relative abundance of class *Actinobacteria* and *Thermoleophilia* which belong to the copiotrophic microbial groups (Zhao et al., 2017) are more inclined to use labile carbon (Wang et al., 2018). Nevertheless, a contrasting trend was noticed in this study. 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 which is in contrast to the study conducted by Skariah et al. (2023) who observed that sand, clay, and silt were significantly correlated with microbial diversity, where sand had a strongest negative correlation whereas clay showed significantly positive correlation. Also, highly significant negative correlations were observed between both classes *Actinobacteria* and *Thermoleophilia* and total sodium.

Members of *Chloroflexota* belongs to oligotrophic phylum and is likely to be more abundant in nutrient-poor soils (Choudhary et al., 2021); thus, higher relative abundance of *Chloroflexota* was found in NSnM and SGIP. Class *Ktedonobacteria* of this phylum are ubiquitous in terrestrial environments, still, our knowledge of their habitat preference and ecological role is limited (Yabe et al., 2017a; Zheng et al., 2019). Typically, they show low abundances in common terrestrial environments (e.g., soil, sand and bark), and can be abundant in extreme environments such as geothermal sediments (Yabe et al., 2017a). However, OTUs belonging to class *Ktedonobacteria* reached a relative abundance of over 9% in soil NSnM (Table 20), which is similar to that of was observed in geothermal sediments by Yabe et al. (2017a). Recently it was suggested by Zheng et al. (2021) that members of the *Ktedonobacteria* lineage have a high cellulolytic potential. Based on a genome-wide analysis it was found that many of these bacteria harbour carbohydrate-active enzymes (e.g., endo- and exocellulases), hinting at their role in cellulose degradation (Zheng et al., 2021). Consequently, the high abundance of these potentially cellulolytic bacteria in soil

NSnM can be explained, since this site was used as a meadow with full and rich vegetation cover, and the decomposing plant material could fuel the ktedonobacterial community here. 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%). Members of the genus *Dictyobacter* have been described only recently, and most of them are able to degrade cellulose and xylan, and do not grow at pH higher than 9 (Wang et al., 2019; Yabe et al., 2017b). Interestingly, members of the class *Anaerolineae* were also abundant in soil SGIP, while playing marginal role in the microbial communities of the other soil samples. Unfortunately, little is known about the role of these bacteria in soils. Zhao et al., (2020b) investigated soil bacterial communities along a salinity gradient in the Yellow River Delta and found that members of the class *Anaerolineae* preferred soils with low-salt content.

The higher abundance of *Gemmatimonadota* in soils NChA and NSnA (10.3% and 13.6%, respectively) could be due to the fact that they prefer neutral pH over acidic pH (Lauber et al., 2008; DeBruyn et al., 2011). This high abundance of *Gemmatimonadota* bacteria is unusual, since globally they comprise ca. 2% of soil bacterial communities (DeBruyn et al., 2011). Although these bacteria are ubiquitous members of soil microbial communities, still little is known about their ecological role. Additionally, investigations conducted by Malard et al. (2019) and Guan et al. (2021), *Gemmatimonadota* dominated in both alkaline and highly saline soils. Also, *Gemmatimonadota* were suggested to adapted to dry environments because they occur in high relative proportions in semi-arid and arid soils and desert (Neilson et al., 2017; Ahmed et al., 2018; Zhao et al., 2020) and more abundant in drought conditions (Ren et al., 2018). DeBruyn et al. (2011) suggested that they are adapted to low-moisture conditions but cannot tolerate moisture fluctuations. Nevertheless, *Gemmatimonadetes* are often reported among the most dominant bacteria in the rhizosphere of maize (Qaisrani et al., 2019; Wen et al., 2016). Moreover, Zhu et al. (2018) observed that sustainable agricultural management practices (e.g., returning all crop-residues to the soil) further increased the relative abundance of members of the phylum *Gemmatimonadota* in the soil microbial community of a maize cropping system. Based on all these it is well explained why *Gemmatimonadota* bacteria showed the highest relative abundances in soils NSnA and NChA.

Similar to *Gemmatimonadota*, members of the class *Phycisphaerae* (phylum *Planctomycetes*) showed the highest relative abundances in soils NSnA and NChA. The first representatives of *Phycisphaerae* were described more than a decade ago from marine alga (Fukunaga et al., 2009), and their role in soil environment is still largely unknown. The stable-isotope probing-based study of Wang et al. (2015) suggested that these bacteria primarily act as heteropolysaccharide degraders



in soils. Thus, it can be speculated that these bacteria benefited from decaying crop residues in soils NSnA and NChA, causing their high relative abundance.

**Table 19:** Relative abundance of major bacterial classes in the soil bacterial communities revealed by Illumina paired-end 16S rDNA amplicon sequencing. All taxa contributing more than 1% abundance were depicted.

Classes	NSnA	NChA	NSnM	AScP	SGIP
<i>Acidobacteriia</i>	1.51%	0.44%	21.55%	0.44%	1.62%
<i>Blastocatellia</i> (Subgroup 4)	8.79%	7.89%	0.20%	11.63%	13.76%
<i>Holophagae</i>	0.65%	2.18%	3.56%	0.80%	0.99%
<i>Acidobacteria</i> Subgroup 6	6.11%	9.22%	0.19%	2.56%	3.13%
<i>Acidimicrobiia</i>	0.63%	0.88%	2.10%	5.23%	6.89%
<i>Actinobacteria</i>	2.16%	2.48%	7.85%	5.54%	3.13%
MB-A2-108	0.41%	1.90%	0.00%	0.92%	0.74%
<i>Thermoleophilia</i>	3.17%	3.47%	9.65%	6.25%	1.41%
<i>Bacteroidia</i>	6.23%	4.41%	1.76%	2.48%	2.42%
<i>Anaerolineae</i>	0.12%	0.38%	0.08%	0.49%	6.21%
Gitt-GS-136	0.16%	0.80%	0.00%	0.83%	1.75%
<i>Ktedonobacteria</i>	0.76%	0.01%	9.37%	0.00%	0.00%
KD4-96	0.48%	0.95%	0.67%	2.48%	2.02%
<i>Entothionellia</i>	0.00%	0.00%	0.00%	1.66%	0.54%
<i>Bacilli</i>	1.98%	1.47%	1.11%	0.70%	9.17%
AKAU4049	0.15%	1.08%	0.00%	1.06%	0.00%
<i>Gemmatimonadetes</i>	12.52%	8.19%	2.01%	2.38%	2.42%
S0134_terrestrial_group	0.76%	0.76%	0.00%	1.00%	1.11%
<i>Phycisphaerae</i>	8.12%	6.92%	2.45%	3.88%	1.82%
<i>Alphaproteobacteria</i>	25.10%	13.98%	14.50%	19.36%	18.11%
<i>Deltaproteobacteria</i>	1.81%	2.80%	2.02%	5.41%	4.60%
<i>Gammaproteobacteria</i>	10.50%	16.41%	6.41%	12.58%	11.63%
NC10	0.16%	2.24%	0.00%	2.30%	0.41%
<i>Verrucomicrobiae</i>	5.27%	3.52%	11.98%	3.22%	1.00%
Others	2.44%	7.64%	2.54%	6.79%	5.13%

Members of the class *Verrucomicrobiae* were most abundant in soil NSnM (12%) and the least abundant in soil SGIP (1%). It is well known, that *Verrucomicrobiota* are ubiquitous in soil, and

the highest relative abundances can usually be observed in soils from humid grasslands and prairies (Bergmann et al., 2011). They tend to thrive in neutral pH environments, with higher numbers observed in high-pH conditions (Bartram et al. 2014). Conversely, their abundance decreases with higher salinity, and higher percentages of *Verrucomicrobia* were observed in low-salt than high-salt soil (Dash et al., 2020). It was also observed by Bergmann et al. (2011) that significantly lower abundances can be observed in soils of arid/semi-arid grasslands and agricultural lands. Our results further confirm this observation.

In a study by Bogati et al. (2023), the relative abundance of *Bacillota* (*Firmicutes*) was increased after prolonged drought conditions which reflects the importance of soil moisture. 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%). Members of the genus *Bacillus* are often the major isolates in studies aiming to cultivate halophilic phosphate-solubilizing bacteria from salt-affected soils (Jiang et al., 2018, 2020). The fact that halophilic *Bacillus* strains often show alkaliphilic characteristics as well explains why *Bacilli* were considerably abundant in soil SGLP, which showed the highest pH value among the investigated soils (Arora & Vanza, 2018).

*Proteobacteria* (*Pseudomonadota*) are commonly found in various soil environments such as rhizospheres, saline soils, and semiarid soils, as reported by Sojka et al. (2003) and as observed in the study conducted by Mhete et al. (2020). Several authors (Fierer et al. 2007; Eilers et al. 2010; Mhete et al. 2020) have observed that the abundance of *Proteobacteria* tends to rise with increased organic carbon availability in soils, which is in contrast from the findings of our study. Within the phylum *Pseudomonadota*, members of *Alpha*- and *Gammaproteobacteria* were dominant in all of the samples. This is similar to the study done by Zhao et al. (2020) who observed that phylum *Pseudomonadota* (*Alpha*- and *Gammaproteobacteria*) was dominant in saline soils. However, in sample NSnA most of the alphaproteobacterial sequences (~19%) belonged to a single operational taxonomic unit (OTU), which was most closely related to the *Sphingomonas parvus/limnosediminicola* lineages (98% 16S rRNA gene sequence homology) (Table 20). Not surprisingly, this sample had the lowest diversity index (Table 21). The same OTU was also abundant in sample NChA, although at a much lower level (~6%). In general, it was observable that genus *Sphingomonas*-related OTUs were overrepresented and characteristic in these two soil samples. The most abundant gammaproteobacterial OTUs were detected mainly in sample AScP and were most closely related to *Collimonas arenae/Glaciimonas singularis* and *Azoarcus olearius*, respectively.

In most of the soil samples the phylum *Acidobacteriota* was represented by members of the class *Blastocatellia*, except sample NSnM, where this group was practically missing. In a study by

Ivanova et al. (2020) the relative abundance of *Blastocatellia* was observed maximum in unfixed sand and declines in vegetative soils. Moreover, in samples SGIP and AScP the most abundant OTUs could be linked to the genus *Brevitalea*. The closest relatives of these OTUs were *B. aridisoli* and *B. deliciosa*, although at a relatively low level of 16S rRNA gene similarity (~93-94.2%) (Table 20). These *Brevitalea*-related OTUs were detectable in all of the soil samples at minimum 2% abundance, except sample NSnM, in which these OTUs were not detectable. In the case of this latter sample, the most abundant *Acidobacteriota* -related OTU (with 4.6% abundance) could be linked to an *Acetobacteraceae*-bacterium (*Acidobacterium ailaui*) within the class *Acidobacteria*. A major group of *acidobacteria* in soils under fully developed plant cover was demonstrated by *Acidobacteriia* in NSnM which seem to specialize in degrading plant-derived organic matter (Ivanova et al., 2020). The most abundant OTU in this sample (4.8% abundance) was a *Ktedonobacterales*-bacterium (distantly related to *Dictyobacter aurantiacus*) within the phylum *Chloroflexota*. Most importantly, these two later OTUs were characteristic only for sample NSnM and missing from other samples. Members of the class *Ktedonobacteria* have been found in a wide range of terrestrial environments, including common soil (forests, gardens, and sand) as well as extreme environments (such as geothermal areas) (Yabe et al., 2017a). The outlying nature of sample NSnM was clearly observable, since several other TOP20 OTUs were also exclusively abundant in this sample. These OTUs could be linked to an *Actinoallomurus*-related bacterium of the phylum *Actinobacteriota* (OTU13, 4%), a *Candidatus Solibacter*-related bacterium of the phylum *Acidobacteriota* (OTU14, 3.9%), a *Chtoniobacteraceae*-related bacterium of the phylum *Verrucomicrobiota* (OTU15, 3.2%), and to *Bradyrhizobium macuxiense* of the phylum *Pseudomonadota* (OTU11, 3%) (Table 20). Members of the class *Bacilli* within the phylum *Bacillota* were abundant only in sample SGIP. Not surprisingly, OTU5, which could be linked to *Bacillus nealsonii* (98.9% 16S rRNA gene homology), was characteristic of this sample (3.6% abundance). Shabaan et al. (2022) observed that halotolerant *Bacillus* helps in improving soil enzyme activities in the rhizosphere under salinity stress. However, the enzyme activities at SGIP were low compared to other salt affected soils. Members of the phylum *Actinobacteriota* were most abundant in samples NSnM and AScP (~19% abundance in both samples) as continuous planting results in a significant change in the abundance of *Actinobacteria* (Liu et al., 2021; Wang et al., 2023), although different lineages were detectable. While in sample NSnM members of the genera *Actinoallomurus* and *Gaiella*, together with a *Solirubrobacterales*-bacterium were abundant, members of the family *Iamiaceae* were the most abundant *Actinobacteriota* -related bacteria in sample AScP. Less copious phyla *Bacteroidetes*, *Entotheonellaeota*, *Latescibacteria*, *Planctomycetes*, *Rokubacteria* and others were also identified in this study. List of the 20 most abundant OTUs with their phylogenetical relationship is presented in Table 20.

**Table 20:** 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	SGIP	NSnA		
1	2.8	2.3	6.2	1.4	18.7	<i>Sphingomonas parvus/limnosediminicola</i>	98.0
2	ND	3.1	3.1	7.5	3.5	<i>Brevitalea aridisoli/deliciosa</i>	93.7
3	ND	4.6	2.6	1.9	1.4	<i>Brevitalea aridisoli</i>	94.2
4	0.3	1.1	3.2	2.2	0.6	<i>Sphingomonas aquatilis/melonis/humi</i>	98.9
5	1.0	0.7	0.8	3.6	1.0	<i>Bacillus nealsonii/oryzisol/circulans</i>	98.9
6	ND	3.3	1.0	0.9	0.5	<i>Collimonas arenae/Glaciimonas singularis</i>	92.3
7	ND	3.3	1.7	ND	<0.1	<i>Azoarcus olearius</i>	91.0
8	4.8	ND	ND	ND	ND	<i>Dictyobacter aurantiacus</i>	86.4
9	4.6	ND	ND	ND	ND	<i>Acidobacterium ailaui</i>	91.6
10	ND	1.1	0.8	2.5	0.3	<i>Halochromatium roseum</i>	89.0
11	3.0	0.3	0.2	0.6	0.4	<i>Bradyrhizobium macuxiense</i>	99.6
12	ND	0.6	1.5	<0.1	1.7	<i>Sphingomonas daechungensis</i>	99.5
13	4.0	ND	ND	ND	ND	<i>Actinoallomurus purpureus/spadix/vinaceus</i>	95.1
14	3.9	<0.1	ND	<0.1	<0.1	<i>Candidatus Solibacter sp.</i>	98.6
15	3.2	ND	<0.1	ND	0.5	<i>Roseimicrobium gellanilyticum</i>	88.8
16	<0.1	1.1	0.7	1.2	0.7	<i>Stenotrophobacter terrae</i>	97.3
17	ND	0.5	2.2	0.5	0.5	<i>Vicinamibacter silvestris</i>	93.1
18	ND	3.1	<0.1	ND	ND	<i>Aquihabitans daechungensis</i>	91.6
19	ND	ND	<0.1	ND	3.2	<i>Nitrosospora lacus</i>	90.3
20	ND	0.4	2.3	0.6	0.2	<i>Methyloversatilis thermotolerans</i>	90.3

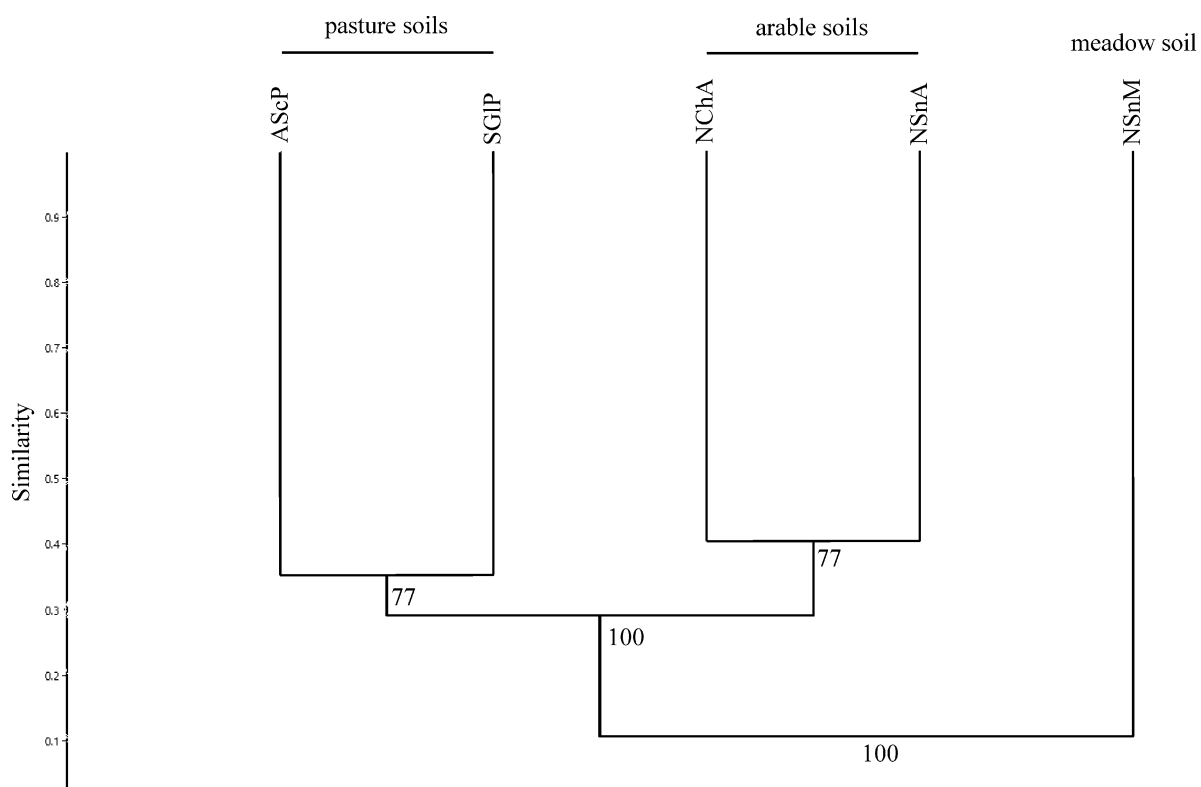
**Table 21:** OTU-based  $\alpha$ -diversity indices of the soil bacterial communities.

Soil samples	$\alpha$ -diversity indexes				
	Sobs	Chao	ACE	Shannon	Inverse Simpson
NSnM	332.2	337.3	335.7	4.86	64.18
AScP	433.4	547.0	496.3	5.05	80.96
NChA	433.0	549.1	504.4	5.11	83.19
SGIP	475.0	562.7	517.0	5.23	78.69
NSnA	404.4	481.5	440.1	4.77	23.97

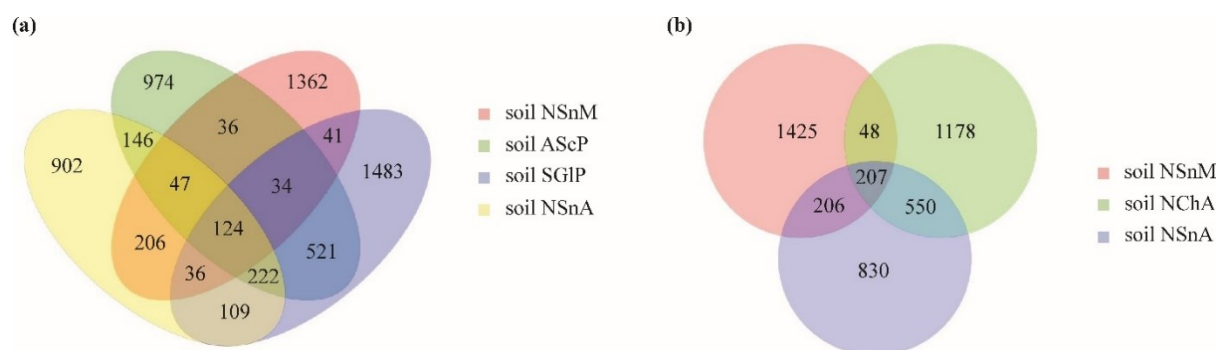
Regarding OTU based diversity indices it was observable, that in case of the Shannon index only small differences were detectable between the samples, thus the Shannon index values ranged between 4.77 (soil NSnA) and 5.23 (soil SGIP) (Table 21). On the other hand, the Inverse Simpson

value, which is influenced by dominance/abundance of OTUs, showed much larger variability. The lowest Inverse Simpson value was recorded in case of soil NSnA (23.97), followed by soil NSnM (64.18). The highest value was recorded in case of soil sample NChA (83.19). Unlike to  $\alpha$ -diversity indices, which showed the lowest values in case of soil NSnA, the species richness estimators (Sobs, Chao and ACE) yielded the lowest value in case of soil NSnM, followed by soil NSnA.

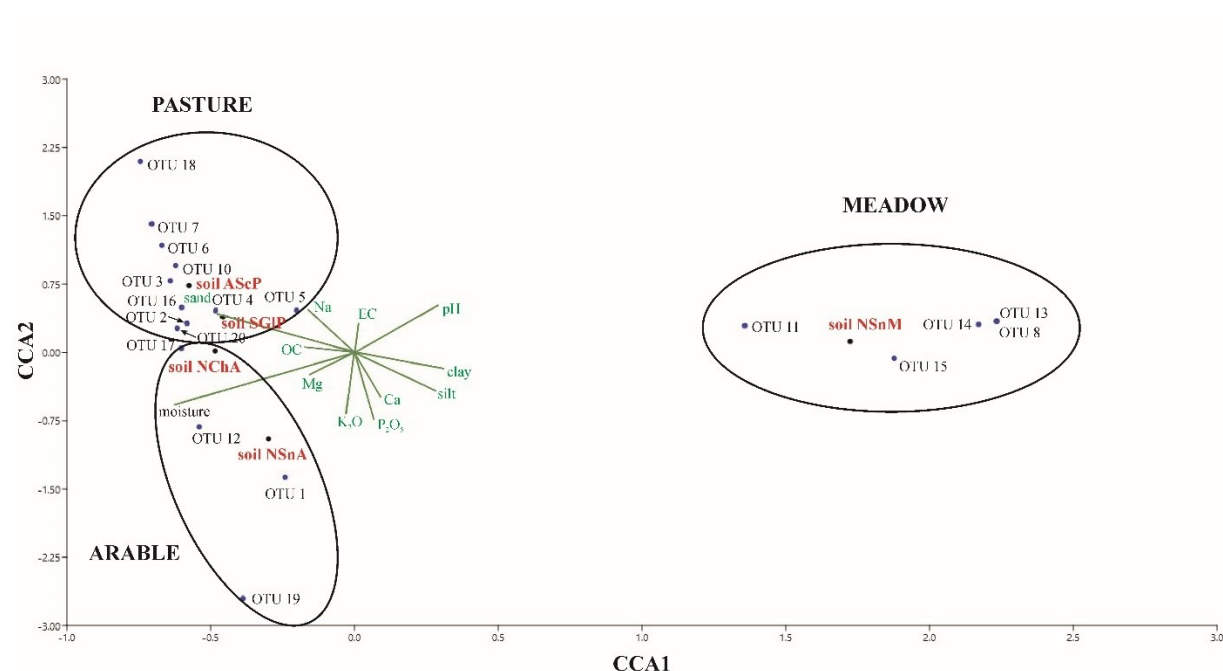
To reveal relationships between the soil bacterial communities, an OTU-based UPGMA dendrogram was created by applying the Bray-Curtis similarity index. On the dendrogram it was clearly observable that bacterial community composition of soil NSnM distinctly differed from that of the other samples, which 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 SGIP 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 Correspondence 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 20) 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 *Ktedonobacterales* bacterium (*Chloroflexota*) (OTU 8), an *Acidobacteraceae* bacterium (*Candidatus Solibacter* sp., OTU 14), a genus *Actinoallomurus*-related bacterium (OTU 13), a "*Spartobacteria*"-related bacterium (OTU 14) and a *Bradyrhizobium*-related bacterium (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, SGIP 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  $\text{Na}^+$  concentration.

## 5. 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, Gleysols 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.

## 6. NEW SCIENTIFIC RESULTS

The effect of land use on chemical properties (organic carbon; pH; electrical conductivity; available P, K, Ca, Mg, Na), microbiological properties (basal soil respiration, microbial biomass carbon, dehydrogenase activity and phosphatase activity), and physical property (moisture content) of salt-affected soils developed under different geographical locations and climate i.e. Hungary and India were studied. Our results proved that the microbiological properties of the soils reflect often, frequent changes in soils, more effectively, than the chemical and/or physical ones.

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.

## 7. SUMMARY

Salt-affected soils (SAS) are widely distributed throughout the world, which is approximately 20% of the global agricultural land. Salinization and sodification affect physico-chemical, biological, and biochemical properties of soil and causing major problems for crop productivity to a significant extent. This has become a severe threat in both places i.e., Hungary and India. An increase in soil salinity results in less microbial and plant growth and salinity beyond tolerance may cause death of microbial cells and degradations of plant tissues which may also affects the biodiversity and carbon storage. There is a close interaction between land use and soil properties as land use practices affect the soil quality, soil functions and ecological processes due to modifications in the physical, chemical and biological properties of the soils.

Thus, it is important to understand the effect of different land use (arable land, pasture land and meadow) on soil physical, chemical and microbiological properties of salt-affected soils (Solonetz and Solonchak) and some slightly-salt-affected soils (Chernozem and Gleysols). Soil microbial activities and community structure were also investigated as soil microbial activity plays a key factor in the biodegradation of organic matter, nutrient cycling, energy transformation, formation of soil structure, and plant growth.

Soil samples were collected from the upper surface layer (0-15 cm depth) from eight plots of 100 m<sup>2</sup> from each site namely AScP, NSnA, NSnM, NChA and SGIP in Hungary and from four plots of size 100 m<sup>2</sup> from bare land (KSnB) and pasture land (KSnP) each and eight plots of same size from arable land (KSnA) in India. Soil physical property (moisture content), chemical properties (OC, pH, EC, P<sub>2</sub>O<sub>5</sub>, K<sub>2</sub>O, Mg<sup>2+</sup>, Ca<sup>2+</sup> and Na<sup>+</sup>), microbiological properties (basal soil respiration, microbial biomass carbon, dehydrogenase activity and phosphatase activity) were investigated. Furthermore, Phospholipid fatty acid (PLFA) and Illumina 16S rRNA gene amplicon sequencing was used to precisely assess the bacterial community composition of the chosen soil samples.

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. Based on chemical properties and moisture content, one-way ANOSIM (Analysis of similarities) proved that sites from Hungary (AScP, NSnA, NSnM) and India (KSnB, KSnP, KSnA) were statistically different from each other. The results of principal component analysis (PCA) showed that soil samples from Hungary and India must be separated unambiguously from each other; furthermore, the Hungarian ones differing in soil type and land use could be also differentiated. Cluster analysis (Bray-Curtis) gave similar results for microbiological properties in Hungarian sites while in Indian sites, three land use practices were

grouped into two clusters where the pastureland was grouped to both arable land and bare land. The Hungarian sites have preferable soil chemical properties which resulted in more favorable microbiological parameters comparing with the Indian sites.

In Solonetz soils, (NSnA and NSnM) the  $\text{Na}^+$  values are lower in comparison to the Solonchak soil (AScP) as the groundwater table is lower (with a level that ranges approximately between 1.5 - 3.0 m from the soil surface), thereby accumulating a low amount of  $\text{Na}^+$  at the soil surface. The values of the microbiological properties (BSR, MBC, DHA and phosphatase) at the Solonchak pasture (AScP) indicated that the AScP plots were microbiologically more active with regard to the largest microbial community, as indicated by the PLFA results.

The cultivated fields have higher nutrient contents due to the regular fertilization processes, but the main macro elements did not affect significantly the studied microbiological parameters. CCA results showed that the variation in microbial activity among the different land use practices was probably associated with the soil moisture level which played an important role in the diversification of microbial activities. However, a PCA indicated the role of  $\text{P}_2\text{O}_5$  in the differentiation of land use types. Moreover, the results obtained in this study showed that the effects of soil properties and management practices had an influence on soil microbial activity and community structure. In contrast to the other soil classification units, which were slightly-salt affected soils, the salt affected soils made a well-separated cluster based on all of the investigated microbiological properties, including phospholipid fatty acid.

Bacterial communities of the investigated soil samples were dominated by members of *Pseudomonadota* (23-37%), followed by *Acidobacteriota* (17-25%) and *Actinobacteria* (7-20%). Members of *Chloroflexi* were abundant in soils NSnM and SGIP (both 11%), while *Gemmatimonadetes* in soils NChA and NSnA (10% and 14%, respectively). Members of *Verrucomicrobia* were abundant highly in sample NSnM (12%), while bacteria belonging to the phylum *Bacillota* were detected in notable amount only in sample SGIP (9%).

Within phylum *Acidobacteriota*, 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. 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. Class *Actinobacteria* and *Thermoleophilia* of phylum *Actinobacteriota* were highly abundant in both NSnM and AScP and were marginal in other samples. The higher abundance of *Actinobacteriota* at Apaj site was characterized by the sandy soil texture and high sodium content. With low soil moisture at SGIP

and due to high sand content, class *Bacilli* belonging to the phylum *Bacillota* were considerably abundant in soil SGIP (9%).

Within the phylum *Pseudomonadota*, members of *Alpha*- and *Gammaproteobacteria* were dominant in all of the samples. However, in sample NSnA most of the alphaproteobacterial sequences (~19%) belonged to a single operational taxonomic unit (OTU), which was most closely related to the *Sphingomonas parvus/limnosediminicola* lineages (98% 16S rRNA gene sequence homology). Not surprisingly, this sample had the lowest diversity index. The same OTU was also abundant in sample NChA, although at a much lower level (~6%). Moreover, in samples SGIP and AScP the most abundant OTUs could be linked to the genus *Brevitalea*. Furthermore, in NSnM samples the most abundant *Acidobacteriota*-related OTU (with 4.6% abundance) could be linked to an *Acetobacteraceae*-bacterium (*Acidobacterium ailaui*) within the class *Acidobacteriia*. OTUs belonging to class *Ktedonobacteria* reached a relative abundance of over 9% in soil NSnM, which is similar to that of was observed in geothermal sediments. The most abundant OTU in this sample (4.8% abundance) was a *Ktedonobacterales*-bacterium (distantly related to *Dictyobacter aurantiacus*) within the phylum *Chloroflexota*.

Moreover, OTU based dendrogram showed 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. Also, results from venn-diagrams revealed the distribution of OTUs among the samples. The highest ratio of shared OTUs (20%) was observed between the arable soil samples NSnA and NChA, followed by the two pasture soils SGIP and AScP (19.2%). Further, the canonical correspondence analysis (CCA) based on the soil abiotic parameters and the abundance values of the TOP20 OTUs showed a distinct separation of soil NSnM from the others.

Our findings demonstrated that, compared to chemical and/or physical soil properties, microbiological properties of the soil reflect often/ frequent changes, more effectively, in the soil. As salt-affected soils and slightly-salt-affected soils are far away from each other in terms of taxonomic distance, soil types were the primary determining factor. For soil groups with short taxonomic distances, land use had more pronounced effects on soil microbiological properties. Furthermore, land usage and soil texture were the key factors which shaped bacterial community compositions of the investigated soils.

## 8. RELATED PUBLICATIONS

### RESEARCH ARTICLE

**R.K. Gangwar**, A. Tánicsics, M. Makádi, M. Farkas, M. Cserhádi, 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) (Q2)

**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) (Q2)

**R.K. Gangwar**, M. Makádi, I. Demeter, A. Tánicsics, M. Cserhádi, 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. Tánicsics, 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)

### CONFERENCES

**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. RTEASTN/Proceeding/2016/109-112 (ISBN- 978-81-932601-6-6) (Conference paper)

**R.K. Gangwar**, M. Makádi, E. Michéli and T. Szegi. Salt affected soil and soil microbiological properties. 2016. Annual meeting of soil science. Debrecen, Hungary, September 1-3, pp. 66. (Abstract)



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