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

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ATS	Atsbi Wenberta
BS	Base saturation
CASCAPE	Capacity building for scaling up of evidence-based best practices for increased agricultural production in Ethiopia
CEC	Cation exchange capacity
cmol kg^{-1}	Centimole per kilogram
CSOB	Csobánc
DNA	Desoxyribonucleic acid
EcoFINDER	Ecological function and biodiversity indicators in European soils
ENVASSO	The Environmental assessment of Soil for monitoring
EU	European Union
FAO	Food and Agriculture Organization
GUF	Gödöllő university forest
GBG	Gödöllő botanical garden
g cm^{-3}	Gram per centimeter cube
HSCS	Hungarian Soil Classification System
HOGR	Hort grass land
HOAR	Hort arable land
ITS	Inter transcribed spacer
IUSS	International Union of Soil Science
JM	Józsefmajor
KAMG	Károly-magaslat
LUT	Land use type
LMH	Laelay Maichew
MC	Soil moisture content
MEA	Millennium Ecosystem Assessment
mg kg^{-1}	Milligram per kilogram
Org C	Organic carbon
OTU	Operational taxonomical unit
PCA	Principal component analysis
PC	Principal component

PCR	Polymerase chain reaction
PGPB	Plant growth promoting bacteria
rRNA	Ribosomal ribonucleic acid
RSG	Reference Soil Group
SiCL	Silt Clay Loam
SMR	Soil microbial respiration
SOC	Soil organic carbon
SOM	Soil organic matter
SZP	Szárítópuszta
SZHE	Szárhalmi forest
TN	Total nitrogen
T-RFLP	Terminal restriction fragment length polymerase
USDA	United State Department of Agriculture
VERP	Verpelét
WRB	World reference base for soil resources
mg CO_2 50 g^{-1}--soil $10days^{-1}$	milligram of carbon dioxide per 50 gram of soil per 10 days

1. INTRODUCTION

This chapter first discusses the background and rationale. It briefly highlights the importance of soil biodiversity, environmental factors influencing soil biota, and selected bioindicators to measure soil biodiversity. Then, the research problem with objectives and justification of the study are presented.

1.1. Background and rationale

Soil biodiversity is known as a vital player in ensuring the functioning of soil and as a supplier of several ecosystem services (Orgiazzi *et al*., 2016). Ecosystem services are defined as 'The benefits people obtained from the ecosystem' (MEA, 2005). Mostly, soil biodiversity plays a key role in nutrient cycling, forming, and maintaining soil structures and water infiltration and purification, which are the bases for other ecosystem services (Jones *et al*., 2008).

Despite the wide range of ecosystem services soil biodiversity provides, it has been under constant threats mainly due to human activity. Currently, biodiversity loss has become a global concern as numerous studies showed that it will negatively affect ecosystem services on which society depends. Land use change is an important form of global pressure affecting biodiversity. Over the last 50 years the land use change has become rapid and more intensified as the demand of food, fiber and biofuel has increased. Globally, it is estimated that between 40 and 50% of land has been transformed or degraded by humans since 1945 (Vandewalle *et al.*, 2010).

Several studies have measured the impact of land management and land use on the diversity and functioning of soil biota. Land conversion, from grassland or forest to cropped land, results in rapid loss of soil carbon, reduce the water regulation capacity of soils and their ability to withstand pests and contaminations (Turbé *et al.,* 2010). Generally, increasing agricultural intensification has shown to reduce soil biodiversity (Tsiafouli *et al*. 2015). Aksoy *et al*. (2017) found that natural diverse vegetation promotes soil biodiversity, while intense mono-cropping maintains only a subset of soil microbes, causing a decrease in biodiversity.

After the Rio Conference in 1992, bioindication has emerged as a useful process for environmental protection, particularly of the soil, which is a complex entity able to perform a multitude of key functions, vital for life. By measuring soil organisms integrally with multidimensional phenomena including time, it is possible at least in principle to ascertain the full potential of a soil to deliver key soil process (Stone *et al*., 2016). Biological indicators have the potential to provide early warning because they can capture subtle changes in land quality as a result of their integrative

nature that simultaneously reflect changes in physical, chemical, and biological properties of the soil.

Biodiversity monitoring programs are often limited to aboveground biodiversity (Gardi *et al*., 2013) or for soil physicochemical properties and various risks factors, such as erosion, compaction metal pollution and desertification (Stone *et al*., 2016). However, indicators related to the decline of soil biodiversity are measured very rarely. For example, only 5 of 29 countries within Europe have monitoring sites for earthworms (Jeffery *et al*., 2010). Currently, The Netherlands (BISQ), France (RMQS), UK (Countryside Survey), and Italy BIO-BIO project are key examples among few national monitoring programs in Europe, at European Union (EU) level, The ENVironmental ASsessment of Soil for mOnitoring (ENVASSO), and Ecological Function and Biodiversity Indicators in European Soils (EcoFINDER) have been known monitoring schemes for soil biodiversity over the last 20 years (Gardi *et al*., 2013).

1.2. Research problem

Numerous studies have been conducted related to assessing soil biodiversity using different methodologies. However, to date research are largely focused on measuring species diversity (e.g., Ibekwe 2002; Fierer and Jackson 2006; Sousa *et al*., 2006) and a few of them assess only the biological functions (e.g., Ponge, 2003; Griffiths *et al.*, 2016). However, both species diversity and biological functions need to be considered when assessing and monitoring soil biodiversity (Creamer *et al*., 2016b). Recently, under European based program '(EcoFINDER)', studies have been conducted aiming to identify and evaluate indicators for soil diversity and ecosystem services across Europe (e.g., Ritz *et al*., 2009; Faber *et al*., 2013; Creamer *et al*., 2016b; Dirilgen *et al*., 2016; Griffiths *et al*., 2016; Stone *et al*., 2016). Among the proposed biodiversity and ecological indicators, earthworm (abundance, biomass, and diversity), microbial respiration, and microbial diversity have been included (Stone *et al.*, 2016). Nevertheless, these indicators have rarely been studied together across a range of soil and land use types (LUTs) in Hungary and Ethiopia. Particularly in Ethiopia, the soil physicochemical properties of major soil types of the country have been well studied while the biological properties have very rarely been investigated (Delelegn *et al*., 2018). Understanding the effects of land use type and soil type on soil biodiversity has paramount importance to monitor the responses of soil ecosystem to global change and to design effective sustainable soil management system. Hence, the purpose of this study was to characterize the surface soils of some major soil groups of Hungary by soil microbial respiration (SMR) and earthworm communities across three LUTs (forest, grassland, and arable land), and Ethiopia by microbial respiration and bacterial community structure on arable land in order to verify whether

there is a significant difference in microbial and earthworm communities in relation to soil types and land use characteristics. Working on the hypothesis that significant difference would be observed on microbial and earthworm communities among different soil and LUTs the following objectives were identified.

1.3. Study objectives

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

1.4. Justification of the study

Currently, sustainable soil management has been gaining global attention to minimize soil degradation (Stockdale *et al*., 2019). Adopting soil management practices that are compatible with the environment is paramount for the maintenance of soil health. Soil health represents the continuous capacity of soil to function as a living ecosystem, depending highly on different ecological processes governed by soil organisms (Dube *et al*., 2019). In this context, the significance of soil biota for the improvement of soil fertility through biological processes becomes a key component of a strategy towards sustainable soil management. Earthworm and microbial communities are important bioindicators to monitor soil fertility because of the vital roles they play in ecosystem functioning (Stone *et al.*, 2016). To adopt management strategies that promote soil biodiversity, first, we need to understand factors influencing their activity, abundance, and diversity. Studies have reported that soil and LUTs are among the major variables that govern the biological component of the soil (e.g., Turbé *et al*., 2010). However, the extreme

spatial and temporal heterogeneity of soils, and the complex interaction among biological, physical, and chemical components of the soil, makes the prediction of soil and land use effects challenging. Although the general principles underline the effects of these factors on soil biota is well understood, site specific information is needed for their interpretation in a local context. Thus, this study provides site-specific information that is important to support the development of management alternatives to maximize and sustain soil functions in the study area.

2. LITERATURE REVIEW

This chapter presents the theoretical base required for this thesis. First, a brief description on ecological roles and major threats of soil biodiversity is presented. Second, the concept of bioindicator and major EU based biomonitoring programs is provided. Third, selected bioindicators for this research (earthworm communities, soil microbial respiration (SMR), and bacterial communities), their contributions to the maintenance of soil fertility, how they are influenced by soil properties, LUT, and agricultural management, and available protocols to measure them are discussed in detail. Lastly, major soil types of Hungary and Ethiopia and an overview of related soil biodiversity studies in Hungary and Ethiopia are presented.

2.1. Introduction

Soil biodiversity is the variation in soil life, from genes to communities, and the variation in soil habitats, from microaggregates to entire landscapes (Turbé *et al*., 2010). 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. One teaspoon of soil contains several thousands of microbial species, several hundred meters of fungal hyphae, and more than one million individuals (Schaefer and Schauermann 1990; Wardle *et al*., 2004).

When considering a broad range of processes that take place in the soil, soil biodiversity may be best considered by focusing on functional groups. Functional groups may define as 'a set of species that have a similar effect on specific ecosystem level biogeochemical or biophysical processes'. According to Turbé *et al*. (2010), soil biodiversity grouped into three all- encompassing ecosystem functions: transformation and decomposition, biological regulation, and soil engineering.

Chemical engineers (transformers and decomposers): organisms responsible for carbon and nutrient transformation through the decomposition of plant residues and other organic matter.

Biological regulators: soil organisms responsible for regulating the populations of other soil organisms through grazing, predation, or parasitism.

Ecosystem engineers: organisms responsible for maintaining soil structure by the formation of pore networks and bio-structures, and aggregation, or particle transport.

2.2. The roles of soil biodiversity

Soil biodiversity is recognized as a crucial player in guaranteeing the functioning of soil and as a provider of several ecosystem services (Orgiazzi *et al.*, 2016). Most of these services are

supporting services or services that are not directly used by humans, but which underlie the provisioning of all other services. These include nutrient cycling, soil formation and primary production (Turbé *et al.*, 2010). In addition to this, soil biota is also involved in most regulatory services, such as atmospheric composition and climate regulation, water quality and quantity, control pest and disease occurrence in agricultural and natural ecosystems, and human disease (Aksoy *et al*., 2017). Furthermore, control and reduce environmental pollution, provide provisioning services directly benefit peoples, for example, genetic resources used for developing novel pharmaceuticals (Turbé *et al*., 2010).

Despite its importance in global ecosystem functioning, the sustainability of agriculture, and the high value of the numerous ecosystem services that it provides, soil biodiversity has often been overlooked in global assessment and mapping studies. One of the reasons could be that soil biota is usually obscured from view and so suffers from being 'out of sight and so out of mind'. Moreover, there is a lack of soil biodiversity data at different scales, and lack of awareness of the value of soil biodiversity (Aksoy *et al*., 2017).

In the last decades, soil quality and soil functions, particularly its intrinsic biodiversity, have become a matter of increasing attention at the scientific and policy levels. Soil quality can be defined as 'the capacity of soil to function as a vital living system to sustain biological productivity, promote environmental quality and maintain plant and animal health'. Even though no legislation or regulation exists that specifically targets soil biodiversity, the European Commission acknowledged the importance of soil biodiversity in the role of ecosystem functioning, stating that "these functions are worthy of protection because of their socio-economic as well as environmental importance" (Evans, 2012). Moreover, the United Nation "Sustainable Development Goals (SDGs)" for the period 2015–2030 has highlighted the importance of soil function as a biodiversity pool, such as habitat, species, and gene. In principle, eight of the revised World Soil Charter, Food and Agricultural Organization (FAO) also acknowledges the fundamental role of soil biodiversity in supporting and safeguarding soil functions and soil ecosystem goods and services (FAO, 2015). Furthermore, the decline in soil biodiversity is identified as one of the eight main soil threats in the EU Thematic Strategy for Soil Protection (Aksoy *et al*., 2017).

2.3. Threats to soil biodiversity

Several studies documented that agricultural intensification and land use change, diminish microbial and faunal abundance and the overall diversity of soil organisms, thereby impairs numerous ecosystem functions, such as nutrient acquisition by plants and the cycling of resources between above- and below-ground communities (Havlicek, 2012; Pelosi *et al*., 2016).

Different authors recognized the connection of soil biodiversity and soil degradation processes. For example, Turbé *et al.* (2010), described the main threats to soil biodiversity as soil degradation, land use management and human practices, climate change, chemical pollution as well as genetically modified organisms, and invasive species. Moreover, Gardi *et al*. (2009) and Orgiazzi *et al.* (2016) added habitat fragmentation, intensive human exploitation, soil organic matter decline, soil compaction, soil erosion, soil sealing, and soil salinization as important threats.

2.4. Indicators for soil biodiversity and major soil biodiversity monitoring schemes in Europe

After the Rio Conference in 1992, soil biodiversity has captured a global attention for the maintenance of soil functions and ecosystem services. Bioindication has emerged as a useful process for environmental protection, particularly of the soil, which is a complex entity able to perform a multitude of key functions, vital for life. In principle, it is possible to determine the full potential of a soil to deliver key soil process by measuring soil organisms integrally with multidimensional phenomena including time (Stone *et al.,* 2016). Ritz *et al.* (2009*)* described biological indicators, by advantage of their involving complex adaptive systems (i.e., the biota) incorporate multi-dimensional phenomena, such as the delivery of key soil processes in ways that other indicators do not. However, a mechanistic understanding of the relationships between soil biodiversity and function, whether in relation to the soil as an ecosystem or as part of a larger ecosystem, are undeniably complex and remain elusive.

Establishing the state of soil biodiversity and evaluating the risks of soil biodiversity loss, requires the development of reliable indicators so that long-term monitoring programs can be set up. Bioindication tools based on a fraction of known soil diversity are certainly imperfect, however, large-scale biological assessment demands huge labor and cost. For this reason, selecting few best groups of indicators is important to serve the purpose of policy making and implementation related to soil quality into the future. But again, bioindication tool selection faces a challenge from compromises between biological and socioeconomic (e.g., effectiveness, cost) constraints. A further challenge is the multi-functional uses of soils and divergent interest, which obstructs progress in regulatory policy (Havlicek, 2012).

Currently, more than 80 methods are relevant as to species diversity or related to biological functions. However, as Havlicek (2012) put, the indicators of soil biodiversity need to be easily applicable to the wide range of stakeholders, including policy makers, farmers, foresters, etc. The value of bioindication should be significant, related to essential ecological functions and have a good correlation with ecosystem processes. The considered parameters must be based on accepted

science, easily available and standardized, to ensure comparability of data among sites and studies. Furthermore, soil biological indicators should be highly sensitive to distinguish differences between several land uses and managements. Generally, any approach to the selection of biological indicators should be objective, realistic, sufficiently flexible to accommodate emergent knowledge and adaptable to changing end-user or policy requirements (Ritz *et al*., 2009). To date, no comprehensive indicator of soil biodiversity exists, that would combine all the different aspects of soil complexity in a single formula and allow accurate comparisons (Turbé *et al.*, 2010).

Numerous reviews and reports have been published on biological indicators, with much emphasis on ecotoxicological perspective. Moreover, most reviews of biological indicators have a strong discipline bias, orientated for example to microbial invertebrate or ecological processes. Since the quality of a soil related to the provision of an appropriate set of soil properties and processes necessary for effective soil function, biological indicators can then be used to assess the status and change in ecological soil properties and processes within a physicochemical context. However, establishing connections between specific soil properties and ecosystem processes is a hard task due to the inherent complexity of natural systems (Ritz *et al*., 2009).

As Stone *et al*. (2016) described, while there are monitoring networks for soil physicochemical properties and soil degradation process such as erosion, compaction, and pollution, indicators related to the decline of soil biodiversity are hardly measured. A few numbers of national monitoring program have been established which include monitoring of soil biodiversity and ecosystem function. Such as The Netherlands (BISQ), France (RMQS), UK (Countryside Survey), and Italy BIO-BIO project. Some EU projects have equally investigated monitoring schemes for soil biodiversity over the last 20 years e.g., ENVASSO, and EcoFINDER (Gardi *et al*., 2013). ENVironmental ASessment of Soil for mOnitoring (ENVASSO) was a project established in 2005, as Scientific Support to Policy (SSP) under the European Commission 6th Framework Program of Research. The project has identified three key bioindicators suitable for monitoring changes in soil biodiversity and representative of three functional levels in soil: (i) abundance, biomass, and species diversity of earthworms – macrofauna; (ii) abundance and species diversity of Collembola – mesofauna, and (iii) microbial respiration. Ecological Function and Biodiversity Indicators in European Soils (EcoFINDERS) was set-up in 2009 by European Commission to support EU soil policy making by providing the necessary tools to design and implement strategies for sustainable use of soils, with a specific focus on soil biodiversity and associated ecosystem functioning. The project has identified a wide range of indicators for biodiversity and ecological functions by logical sieve approach, proposed by Ritz *et al*. (2009), that allows a structured discrimination of biological

indicator methods through a series of queries regarding their potential within monitoring. The top ten indicators included three indicators of biodiversity ('Bacteria and archaea', 'Fungi', and 'Mites') by various methods of measurement, and three indicators of ecological function (multiple enzyme assays, multiple substrate-induced respiration profiling, and 'functional genes by molecular biological means'). For this study, earthworm (abundance, biomass, and species richness), bacterial community structrue, and SMR were selected. The following sections discuss the ecological roles of these bioindicators, available methods to measure them, and the influence of LUT, soil propeties, and agricultural management on them.

2.5. Selected bioindicators

2.5.1. Soil microbial communities

The soil ecosystem is a reservoir of diverse microbial communities. It is evident that these soil microbial communities play key roles in several ecosystem services such as soil formation, erosion control and nutrient cycling (Castañeda and Barbosa, 2017). The microbial fraction of the soil is a really essential part of soil fertility as soil microbes highly influence soil metabolic activities. Microorganisms are generally considered the driving force behind litter decomposition processes. They act as both a source and a sink of available nutrients and play crucial roles in various ecosystem functions, such as aggregate formation, nutrient mineralization/immobilization, carbon humification, and degradation of pollutants (Creamer *et al*., 2016a; Zhang *et al*., 2017a). Thus, understanding of the response of microorganisms to environmental parameters is crucial to crop productivity and long-term sustainability of a soil in agricultural ecosystems (Hargreaves and Hofmockel, 2014).

Microorganisms, more than any other organisms, are highly adaptable to varying conditions and respond rapidly to changes (Hargreaves *et al*., 2015). Soil microbial community is a sensitive indicator for changes in land use. The change in total organic matter can be detected early by measurement of soil microbial biomass, long before changes in total soil C or N can be reliably detected (Zhang *et al*., 2017a). For these reasons, they can be considered as reliable indicators of soil health and therefore they are usually used for soil status monitoring.

Soil is extremely heterogeneous because of multiple chemical and physical factors, plus inputs from plants and animals, consequently great variability of microorganisms both in temporal and spatial scales. For instance, it is estimated that the total bacteria diversity present in soil is ranging 4000 to one million separate bacterial genomes per gram of soil (Dequiedt *et al*., 2009). Various studies at plot scale documented significant effects of abiotic and biotic factors on the distribution and composition of soil microbes. Nonetheless, the reported evidence has not been conclusive in

determining the main drivers of microbial distribution. The mechanisms that govern the spatial distribution of microbial communities at larger scales are not well understood because environmental regulators of soil microbes substantially vary at different special scale and different ecosystems (Xue *et al*., 2018). For instance, soil properties and land use were the main drivers of microbial variation compared to topography and climate in some regions of France (Dequiedt *et al*,. 2009). However, Chen *et al*. (2015) noted a strong influence of precipitation and soil factors on the biogeographical variation of soil microbes across grasslands on the Mongolian Plateau in China.

2.5.1.1. Major soil bacteria phyla and their ecological roles

Molecular investigations have reviled that more than 100 phyla of bacterial community exist, of which, less than 10 phyla are abundant in soil (Aislabie and Deslippe, 2013). Although the abundance of major bacterial phyla varies between different soils, *Proteobacteria*, *Acidobacteria*, and *Actinobacteria* are widespread and often abundant groups of bacteria. Phylum *Proteobacteria* is the largest group of gram-negative bacteria that comprises several subphyla; alpha, beta, and gamma are the most commonly found subphyla in the soil (Hugenholtz, 2002). They are predominantly found in environment where resource availability is high, e.g., rhizosphere soils. They are of great biological importance because they include the majority taxa that play key roles in the carbon, sulfur, and nitrogen cycles (Hirsch and Mauchline, 2015). The *Alphaproteobacteria* include nitrite oxidizers such as *Nitrobacter*, *Nitrospira*, *Rhodobacter*, and *Rhodospirillum*; symbiotic nitrogen fixers including *Rhizobium*, *Mesorhizobium*, and *Bradyrhizobium*; methane-oxidizers such as *Methylobacter* and *Methylophilus*; toxic compound degraders e. g., *Sphingomonas*. The *Betaproteobacteria* include major players in carbon turnover such as *Acidovorax*, *Burkholderia*. Members of *Burkholderia* are also known nitrogen fixing and plant growth promoting bacteria. An example of a methanotroph belonging to the *β-Proteobacteria* is *Methylomonas*. The phylum also includes the ammonia oxidizer, *Nitrosospira*, and the iron oxidizer, *Thiobacillus*. The *Gammaproteobacteria* include *Pseudomonas*, the most nutritional versatile group of bacteria that grow on more than 50 different substrates, and sulfur reducer such as *Thiocapsa* and *Chromatium* (Aislabie and Deslippe, 2013). *Acidobacteria* are among the most widespread and abundant bacteria on the planet, however, very little is known about their metabolic capabilities as they are poorly represented in soil culture collections (Kielak *et al*., 2016). *Acidobacteria* metabolize a wide range of simple and complex carbon sources. This phylum also includes known bacteria which able to reduce nitrate to nitrite such as *Acidipila rosea* (Okamura, *et al*., 2011) and *Granulicella mallensis* (Männistö *et al*., 2012), and iron reducer, *Geothrix fermentans* (Coates *et al*., 1999), that can use nitrate as an alternative electron acceptor

(Aislabie and Deslippe, 2013). *Acidobacteria* are known to thrive in acidic environments and resistance to pollutants like uranium and petroleum compounds (Barns *et al*., 2007). *Actinobacteria* are gram-positive bacteria, more abundant in soils than other media, especially in alkaline soils. Members such as *Cytophaga*, *Rhodothermus*, *Salinibacter* play an important role in the decomposition of cellulose and chitin. *Actinobacteria* are well-known as secondary metabolite producers, hence of high pharmacological and commercial interest, especially from the genus *Streptomyces* (Aislabie and Deslippe, 2013).

2.5.1.2. Influence of edaphic factors, land use change, and management on soil microbial biomass, abundance, and diversity

"Land-use is the sum of land utilization categories (e.g., field cropping, grassland management, forestry etc.), the crops or other plants sown or planted in the given site(s) and the modes of their production" (Birkás *et al*., 2012). "Land management means the approach taken to achieve a land use outcome - the 'how' of land use (eg cultivation practices, such as minimum tillage and direct drilling" (Australian Collaborative Land Use and Management Program, 2019).

Land use change and agricultural management may have important effects on soil microbial diversity through changing the physical and chemical properties of the soil. Studies showed that addition of organic matter in soil shifted the microbial community structure from bacterial dominated to fungal dominated (Guo *et al*., 2019). Paula *et al.* (2014) have reported that conversion of primary forest to long-term pasture has changed the microbial functional diversity, specifically the genes related to carbon and nitrogen cycling in Amazon soils. Moreover, their study noted the homogenization of bacterial communities in converted pasture lands despite of the increment of local taxonomic and phylogenetic of soil bacteria diversity, implying a net loss of bacterial diversity. Similarly, Montecchia *et al*. (2015), reported that the alpha diversity of bacterial communities showed an overall increase from forest to long term agricultural system whereas the beta diversity was significantly reduced, suggesting agriculture leads to homogenization of soil bacterial communities over time. Similarly, Ding *et al*. (2013) found that the variation in bacterial community composition was significantly reduced when shrubland was converted to alfalfa fields and the effect of land use was taxonomic group dependent. In other study, Zhong *et al*. (2007), reported that application of some fertilizers for 13 years, the microbial diversity decreased to the point that the community similarity had reached up to 75–85%. Edaphic factors thought to have influence and are important environmental filters shaping soil microbial communities (Hargreaves *et al*., 2015). Various studies documented that soil pH is a strong driver for the variation of soil bacteria diversity and community composition at local, regional, and continental scales (Fierer *et*

al., 2012a; Fierer and Jackson 2006; Montecchia *et al*., 2015; Paula *et al*., 2014). Bacterial diversity is higher in soils with near-neutral soil pH compared to more acidic or more basic soils (Rousk *et al*., 2010). This is because low pH can denature protein and high pH may inhibit microbial growth (Alele *et al*., 2014). In their study Kuramae *et al*. (2014) reviled that C:N ratio in the soil was the main factor that explained different microbial community functional structures. However, land management had no effect on the functional gene diversity in different soils. Other factors, such as nutrient availability, soil temperature, and moisture have also been shown to shape the composition of soil microbial communities (Fierer *et al*., 2012b). Moreover, aboveground plant communities add another level of complexity to the edaphic factor structuring soil microbial communities. The quantity and quality of plant residues together with the chemistry of root exudates inhibit or stimulate certain groups of microbes, thereby resulting distinct microbial communities associated with specific plants (Hargreaves *et al*., 2015).

Soil type has been shown to be one of important variable determining the distribution and species composition of soil microbes (Brockett *et al*., 2012). Xue *et al*. (2018) observed that soil samples dominated by Vertisol had a higher abundance of the belowground communities compared to other soil types. Nacke *et al*. (2011) have documented that soil bacteria diversity and community structure were significantly affected by LUT (forest and grassland), and soil pH had strong influence on bacterial community structure. However, management type (management in each LUT) and other soil properties had minimal impact on soil bacterial diversity and community structure. Conversely, Girvan *et al*. (2003), showed that soil type, particularly soil chemistry, rather than management practices had a prominent effect on total bacterial community compositions. Likewise, on Australian agricultural soils, Wakelin *et al*. (2008) found that soil type was the primary factor determining microbial community structure and catabolic function. Using a sequencing approach, Lauber *et al*. (2008) found that soil pH was a best predictor of bacterial community composition across LUTs, while fungal community composition was more governed by the nutrient status.

Different bacterial communities in the soil may respond differently to land use change. For instance, *Delta-* and *Betaproteobacteria* classes showed highest differences in bacterial community structure across the land use whereas *Alpha-* and *Gamma*proteobacteria displayed rather strong conservatism, indicating that they were insensitive towards land management practices (Wolinska *et al*., 2017). Although the relationship between diversity and functioning is still debatable, it is generally accepted that the more diverse the microbial community, the more

resistance and resilient to environmental perturbations, and therefore more capable of sustaining soil functions (Montecchia *et al*., 2015).

Whether abiotic variables or microbial community structure better predict the ecosystem processes, such as organic C or N mineralization is still a matter of debate. Substantial body of literatures have been documented the paramount importance of abiotic factors governing carbon mineralization and this control was significantly greater than the role played by microbial communities (Wakelin *et al*., 2008). The possible explanation of the lack of relationship between ecosystem processes and microbial communities is the high degree of functional redundancy within microbial communities. However, the methodological approaches used by these studies to investigate the relationship between microbial diversity and processes were either serial dilutions of microbial suspension or by differential fumigation, which preferentially remove the least abundant species or the species most sensitive to fumigation. This may homogenize the microbial diversity to the same active microbial groups, resulting similar level of activity (Nunan *et al*., 2017).

In their study, Nunan *et al*. (2017) noted that environmental context shapes the relative contribution of species to community functioning. In environment, where the activity of microbes was not restricted (organic layer, rhizosphere, and litter layer), C mineralization was related to the composition or diversity of resident microbial communities. However, in case of mineral soils the rate of C mineralization was more influenced by abiotic variables, as all communities mineralize at the rate at which the abiotic constraints allow, suggesting the composition or diversity of the microbial communities may no longer be significant in determining the rate of C mineralization (Lange *et al*., 2014). Plant communities (diversity and functional groups) may influence the performance and shape of soil microbial communities through biomass production, litter quality, seasonal variability of litter production, root-shoot carbon allocation and root exudates (Mitchell *et al,*. 2010). It is well known that resource availability and niche differentiation are enhanced by increasing plant diversity, leading to diverse microbial communities in the soil (Lange *et al*., 2014). Studies documented that plant communities producing litter with high C:N ratio favor decomposition by fungi and vice versa. Moreover, gram-negative bacteria mainly root-associated and thus decompose organic molecules of low molecular weight. On the other hand, gram-positive bacteria can decompose more complex molecules, such as soil organic matter and litter (Kramer and Gleixner, 2006).

Anderson *et al.* (2017) investigated the impact of tillage and depth on microbial community structure (fungi and bacteria) in a long-term field trial (12-years, Lincoln, New Zealand),

discovered the significant variation in microbial community composition among treatments on surface soil samples (0-7.5cm). The variation was more pronounced in bacterial community than in fungi community, suggesting that fungal communities were more adapted to tillage treatment than bacterial communities. However, the tillage effect on microbial composition declined with depth and tillage did not affect the richness of soil microbial communities.

2.5.1.3. Molecular techniques in microbial ecological studies

Although the aspects and characters of soil microbial diversity have been known for decades, it was just a few years ago that the ongoing development of high throughput molecular techniques have made it possible the detailed characterization of taxonomic, phylogenetic, and functional diversity of soil microbial communities unimagined previously (Fierer *et al*., 2012a). Different studies show that less than 1% of soil microbial diversity is culturable. Due to this, culturing methods have only been able to show us the tip of the iceberg of the actual diversity of soil microbial communities (Thies, 2007). Recently, molecular approaches have been widely applied in microbial ecology studies to survey the total microbial community within the complex soil matrix. The advanced molecular techniques including metagenomics and PCR finger printing techniques opening the 'black box' of microbial life in the soil, allowed us to access and characterize the largely undescribed 90-99% of the soil biological community (Thies, 2015). Unlike culturing methods, molecular techniques in microbial ecology studies focus on nucleic acids rather than the ability of forming colonies on laboratory media to investigate microbial communities (Osborne, 2007). The aim of molecular approaches is to extract and characterizing nucleic acids and other cell components, such as phospholipid fatty acids and proteins, thereby describe population diversity as described by taxon richness and evenness (Thies, 2015).

Molecular techniques either detect and quantify individual target organisms or function or analyze the diversity of whole communities of organisms or their functional groups (Elphinstone *et al*., 2018). PCR fingerprinting techniques generate a profile of microbial communities based on target sequences that are phylogenetically or functionally significant. Most widely used taxonomic barcode markers within bacteria and archaea is the gene encoding small subunit of ribosomal RNA, 16S rRNA gene, highly conserved regions but at the sometime sufficiently polymorphic, to provide phylogenetic information (Osborne, 2007). Other commonly used targeted genes are 18s rDNA gene for eukaryotes and intergenic transcribed regions ITS1 and ITS2 to identify soil-borne fungi and oomycetes (Elphinstone *et al*., 2018). Functional genes, such as nifH to compare populations of nitrogen-fixing bacteria or amoA to study ammonia-oxidizing bacterial populations in soil also used predominantly by ecological studies (Thies, 2007).

Molecular finger printing methods have been proven to be powerful tools to examine the response of microbial communities to environmental change and how microbial communities change over time. They also enable us to examine the variations of 'fingerprints' in multiple soil samples simultaneously. Most commonly used techniques include Density Gradient Gel Electrophoresis (DGGE), Temperature Gradient Gel Electrophoresis (TGGE), and Terminal Restriction Fragment Length Polymorphism (T-RFLP) (Fakruddin and Mannan, 2013). These techniques separate PCR products based on fragment lengths and the formed bands in gel electrophoresis are used for community comparisons (Thies, 2015).

Other powerful molecular technique that has been capturing the attention of molecular studies nowadays is metagenomic. Metagenomic is the application of modern genomic techniques which study the genetic material of microbial communities directly from environmental sample i.e., without culturing individual members. Unlike phylogenetic surveys, mostly based only one gene, for instance the 16S rRNA gene, metagenomic give a wider description of microbial communities as it provides to access the functional gene composition of microbial communities (Thomas *et al*., 2012). The understanding of soil microbial taxonomy and phylogeny have been expanding, however, the understanding of how the functional genes encoded in their collective genomes act to structure communities across environmental gradients is still lacking (Fierer *et al*., 2012b). The advantage of metagenomic over PCR fingerprinting methods is that it does not require an initial PCR step, thus it is free of potential PCR biases associated with formation of PCR artefacts and variation in amplification efficiency between different primers (Elphinstone *et al*., 2018). Different techniques are used to extract metagenomic DNA, however these extraction methods do not provide a uniform and unbiased subsample of metagenomic DNA, therefore, it is hard to accurately determine the microbial diversity in the soil (Delmont *et al*., 2011). The level of accuracy of the metagenomic diversity is determined by the amount of metagenomic DNA recovered from the soil. However, no one protocol can provide an accurate determination of species distribution, hence adopting a range of extraction methods that rare species are captured is important (Delmont *et al*., 2011).

Metagenomic approach has revolutionized and widened our understanding of microbial diversity and function. However, the construction and screening of soil-based libraries is difficult and challenging considering the complexity and heterogeneity of the biotic and abiotic components of soil ecosystems (Daniel, 2005). In addition, bio-chemical contaminants, such as humic acids and DNases, make DNA extraction from soils, and subsequent procedures, hard. On top of that recovery of microbial soil DNA that represents the resident microbial community and is suitable

for cloning or PCR still an important challenge (Sabree *et al*., 2009). Hence, the mentioned challenges necessitate further advances in sequencing technologies and bioinformatic tools to handle the enormous amount of data produced (Daniel, 2005).

Next generation sequencing (NGS) method is a novel metagenomic technique that allows parallel sequencing of thousands to millions of molecules simultaneously at low cost with high speed (Raza and Ahmad, 2016). The advancement in NGS has revolutionized the field of microbiology by revealing what has been termed the ‘‘rare biosphere’’ and provided more accurate genomic information of a vast number of microbial communities from a wide range of habitats (Rastogi and Sani, 2011). Illumina-based 16S rRNA gene sequencing, particularly MISeq platform, has recently gained popularity for microbial ecology studies due to its lower costs, higher sequence quality, great flexibility, and high throughput. To minimize cost, however, different community samples are sequencing together in a single Hiseq lane or Miseq run via the use of barcodes, which cause low sequence diversity and unbalanced base composition in a template DNA that ultimately affects sequence output, quality, and error rate (Wu *et al*., 2015). Another challenge of Illumina sequencing is that base-call accuracy decreases with increasing read length due to under or over incorporating of nucleotides, or failure of block removal in a given sequencing cycle (Voelkerding *et al*., 2009).

The first step in all NGS platforms is DNA library preparation which encompasses DNA fragmentation, size selection, and adaptor ligation. The next step involves library amplification of each DNA fragment either by the attachment of DNA fragment to microbeads or on glass slides, in case of illumine sequencing. This eventually leads to sequencing reaction, imaging process, and data analyses (Raza and Ahmad, 2016). For NGS of 16S rRNA gene, after extraction of DNA, a specific region of 16S rRNA gene is amplified and sequenced. The generated sequences then identified based on similarity to reference 16S rRNA gene sequences available in public database (Voelkerding *et al*., 2009).

2.5.2. Soil microbial respiration (SMR)

2.5.2.1. Definition and importance of SMR

SMR is a biological process that converts soil organic matter into atmospheric CO_2, in which soil microflora plays a major role (Creamer *et al*., 2016a). It is an important indicator of soil health as it reflects the level of microbial activity, which is a key factor in mineralization and organic matter decomposition. SMR also relates to soil microbial properties such as microbial biomass and microbial composition (Józefowska *et al*., 2017). Hence, this indicator will give a measure of soil biological functioning (Jones *et al*., 2008). Moreover, SMR is strongly linked to plant metabolism,

photosynthesis and litterfall since the activity of soil microbes is controlled by substrate availability (Semenov *et al*., 2019). However, high SMR may also indicate loss of soil organic matter due to excessive tillage or other soil degradation process (Chen *et al*., 2015). SMR can be measured either by introducing various forms of organic substrate in the soil, substrate induced microbial respiration, or without the addition of organic substrates, basal respiration, under controlled laboratory conditions (Creamer *et al*., 2016a). Measuring SMR in the different environmental conditions has great importance to understand the global climate change as well as soil-gas dynamics that affect soil fertility and plant growth (Lazik *et al*., 2019).

2.5.2.2. Effects of soil and LUTs, and management on SMR

Previous studies have documented the effect of multiple factors on the catabolic functional capacity of the microbial community including precipitation, temperature, soil properties, LUT and management. In their study, Creamer *et al*. (2016b) found that soil properties, pH, organic carbon content (Org_C), total nitrogen (TN) and cation exchange capacity (CEC), had significant effect on substrate utilization by microbes. Similarly, LUT was significant in the discrimination of microbial activity of soils, where grassland sites showed significantly greater substrate utilization compared to arable. In accordance with this finding, Zhang *et al*. (2017a) reported that the previous land use exerted a significant impact on soil microbial biomass after tree plantations. By comparing soil bacteria physiological profiles across a range of grassland sites in The Netherlands and Europe, Rutgers *et al*. (2016a) pointed out that soil management was a significant effect on the activity of microbial communities. Moreover, soil type also found to be another driver for the variation of SMR. For instance, Vasenev *et al*. (2016), investigated the spatial variability of soil respiration per soil type in Mosco region and found that different soil types presented different basal respiration with the highest values for the Luvic Chernozems and lowest ones for Dystric Histosols and Eutric Luvisols although it was not significant due to the large variability. Similar to this finding, Maková *et al*. (2011) monitored the amount of microbial biomass carbon and respiration activities in four of the most widespread soil types (Chernozems, Luvisols, Planosols, Cambisols) in Slovakia used as arable soils and pasture grassland soils and revealed that the basal respiration was not significantly affected by soil type.

García-Palacios *et al*. (2015) found that microbial community abundance and composition were essential for the mineralization of soil organic carbon (SOC) in the presence of labile C. In agreement, Zhang *et al*. (2015) showed that turn over and fate of SOC can be altered by a gradual shift in the dominant species of microbial communities due to interactions between soil microbial community composition, SOC accumulation, and aggregation. However, Guo *et al*. (2019)

investigated the long-term effects of fertilization on soil properties and microbial communities and their relationships with C mineralization and they found that the alterations in soil microbial abundance and community composition, did not significantly influence the C mineralization.

Soil physical and chemical properties significantly change with soil depth (Liu *et al.*, 2018a), hence, it is expected that different soil layers may have distinct microbial communities that are adapted to a specific microenvironment of the soil that could contribute to the variation of SMR along soil depth. It was estimated that 35–50% of the soil microbial biomass found in subsurface horizons (van Leeuwen *et al.*, 2017). Therefore, it is important to investigate SMR across various depths of the soil to understand how microbial decomposition could be influenced by edaphic factors associated with soil depth.

Generally, as soil respiration is the largest CO_2 flux from the terrestrial environment, understanding the response of microbial respiration to the changing environment has implications for global climate change as well as soil-gas dynamics that affect soil fertility and plant growth (Lazik *et al.*, 2019).

2.5.3. Earthworms

2.5.3.1. Ecological roles and functional groups of earthworms

The fundamental role of earthworms in the formation of soils was already largely acknowledged by Darwin (1881): “It may be doubted whether there are many other animals which have played so important part in the history of the world, as have these lowly organized creatures”. Their role in pedogenesis and soil profile development is significant because they can ingest between 2 and 30 times their body weight in soil per day (Boyer and Wratten 2010; Bertrand *et al.*, 2015). Earthworms are considered as ‘ecosystem engineers', as they have a large influence on soil physical, chemical, and biological properties. Furthermore, earthworms play a main role in modifying soil processes by burrowing, moving particles within and between horizons, forming and disintegrating aggregates, and changing porosity, aeration and water infiltration and retention capacity (Blanchart *et al*., 1999). For instance, the elimination of earthworm population due to soil contamination can reduce the water infiltration rate significantly, by up to 93% in some cases (Turbé *et al*., 2010).

Among soil organisms, earthworms are of particular interest to evaluate adverse effects of contaminants. Measuring the earthworm biomass and different earthworm species present allows good evaluation of soil quality. Earthworms possess several qualities required in animals used for biomonitoring of terrestrial ecosystems. They are numerous, easy to sample, widely distributed

and relatively stationary; they are in full contact with the substrate in which they live and consume large volumes of this substrate. Moreover, because of their strong interaction with soil, earthworm populations are also profoundly affected by agricultural practices, such as soil tillage, crop residues, the use of fertilizers and pesticides, etc. (Turbé *et al*., 2010).

Taxonomic indices or functional groups defined a priori, such as eco-morphological groups of earthworms are commonly used to assess the impacts of anthropic activities on soil organisms (Bengtsson *et al*., 2005). However, these methods are associated with some drawbacks. For instance, in case of taxonomic indices, such as species richness, assigning identical weight to each species in the analysis is the main pitfall of this approach. Variation in the definition of the functional group and discrete functional differences between taxa are considered as main drawback in case of a priori functional group approach. Currently, the functional treat-based approach is growingly used in soil ecology to deal with the mentioned drawbacks by drawing causal relationships between individual properties and environmental gradient. Earthworms have been traditionally classified into three functional groups, representing different traits in the soil system. i.e., dwellers in the mineral layer (endogeics), dwellers in the litter layer (epigeics) and vertical burrowers (anecics) (Rutgers *et al*., 2016b). According to Boyer and Wratten (2010), there is little functional redundancy between functional groups of earthworms as each effect the soil nutrient dynamic and structure differently. As a result, the impact of all groups is not the reflection of the sum of individual contribution but is synergistic.

2.5.3.2. Earthworm extraction and identification methods

Reliable, efficient, and quantitative extraction methods are indispensable for field population studies of earthworms. It has been shown that hand sorting, heat extraction with the Kempson apparatus and wet sieving are the most effective techniques for recovering and enumerating earthworm populations. Despite the availability of international standards earthworm sampling, results from different studies are not comparable due to lack of standardization in the sampling protocol. The hand sorting extraction method is time consuming and tends to under-estimate the number of smaller earthworms, juveniles, and cocoons. The alternative way is to use chemicals, such as formalin and mustard oil. However, the extraction capacity of this method is less compared to hand sorting. In addition, it tends to bias sampling towards over-estimating anecic species that have burrows opening directly onto the soil surface and under-sample endogeic species, which cannot surface easily, and epigeic species, which laterally migrate out of the sampling area in response to the chemical. Moreover, formalin is a toxic chemical which poses undesired side effects on vegetation and on other components of the soil fauna. Another alternative method is

electrical extraction. It has an advantage in terms of minimizing soil disturbance but requiring specialized and expensive equipment. Recently, novel tagging techniques using a commercially available, visible implant elastomer, to mark earthworms was developed. This method provides promising opportunity to monitor earthworm populations in their natural environment over long time periods, at least up to a year. Nevertheless, difficulties of using this method with small sized individuals and certain ecological groupings could lessen its applicability. Using a combination of these methods significantly increase the accuracy of the estimate of the earthworm population (Bartlett *et al*., 2010).

To date, researchers predominantly use morphological methods to identify earthworm species. But the process is time-consuming, labor intensive and requires trained specialists. Consequently, sample turnaround is slow. Recently, molecular approaches in the research area of earthworms have been gaining attention and recognition. The use of molecular markers in earthworm research offers the potential additional benefits of identifying previously unknown mysterious species and unlike morphological keys, allow trustworthy identification of juveniles, thus delivering a comprehensive assemblage composition. However, molecular techniques would also need to demonstrate that their reliability and sensitivity in testing multiple populations from large geographical areas (Dupont, 2009; Bartlett *et al*., 2010).

2.5.3.3. Effects of soil and LUTs, and management on earthworm communities

LUT and soil properties can impact massively the soil ecosystem. Earthworms and other soil organisms can be influenced directly or indirectly by these changes (Rutgers *et al*., 2016b). Several studies reported that earthworms are generally more abundant on grassland soils than forest and arable soils, primary due to high food availability and less intensive soil cultivation (e.g., Cluzeau *et al*., 2012; Varga *et al*., 2018). Conversely, Sankar and Patnaik (2018) reported a high earthworm density in forest compared to grassland. By studying earthworm communities across most widespread soil types in Estonia, Ivask *et al*. (200), found a significant effect of soil type on earthworm abundance. Certain soil properties have been repeatedly shown to influence the distribution and composition of earthworm communities. Among these are pH (Moore *et al*., 2013), soil organic matter (SOM) (Bertrand *et al*., 2015), soil C:N ratio (De Wandeler *et al*. 2016), and soil texture (Hendrix *et al*., 1992). Overall, Rutgers *et al.* (2016b) described that land use, vegetation, soil texture, organic matter and soil pH which are known to strongly affect earthworm communities in Europe.

Earthworms are considered as important beneficiary organism in agroecosystem in terms of crop growth, however, their contribution to agroecosystem is largely depended on management (Fonte

et al., 2010). It is well known that conventional tillage affects the earthworm population dynamics, due to the extensive and frequent disturbance on earthworm population (Bertrand, *et al*., 2015) or by associated reduction of organic matter content in the soil (Bartz *et al*., 2014). Conventional tillage also destroys earthworm burrow, modifies resource availability, change soil physical properties (temperature, moisture, and structure) of the soil. Although growing numbers of literature documented the detrimental effects of conventional tillage on earthworm populations (e.g. Ponge *et al*., 2013; Smith *et al*., 2008; Spurgeon *et al.*, 2013), few other studies reported a neutral even positive effect of conventional tillage on earthworm communities (e.g., Bartz *et al*., 2014; Pelosi *et al*., 2016).

2.6. Description of major soil types of Hungary and Ethiopia

2.6.1. Major soil types of Hungary

The traditional Hungarian Soil Classification System (HSCS), established during the 1960s, was based on genetic principles of Dokuchaev (Michéli *et al*., 2019). The highest level, main soil type, comprises 9 soil groups, primarily determined by important pedogenetic processes. In the next higher hierarchical level, 39 soil types are identified. Recently, the demand of global harmonized soil information necessitated the importance of harmonization of the existing national soil classifications with the international systems (Láng *et al*., 2013). Accordingly, the new modernized, diagnostic based Hungarian Soil Classification System, was developed by Michéli *et al*. (2019). This system harmonized the HSCS with World Reference Base for Soil Resources (IUSS Working Group WRB, 2006). The definitions and the limits of the diagnostic horizons and properties are similar with WRB, but much simpler, and adopted for the environmental setting of the Carpathian Basin (Michéli *et al*., 2019). The system defined 15 soil types among which the following are the major ones.

Steppe soils: are well recognized by the traditional HSCS due to the influence of Dokuchaev Chernozem concept. These soils typically characterized by dark, high organic matter rich mineral surface horizon and mostly subsurface horizons with secondary carbonates. In the Carpathian basin, most of the steppe soils are formed on loess and loess-like sediments (Michéli *et al*., 2019) and mostly occur in the North Hungarian Mts., the Transdanubian Mts., the Transdanubian Hills and the Foothills of the Alps (Mezősi, 2017). Chernozems, Kastanozems, and Phaeozems are the correlated WRB reference soil groups (RSGs) (Michéli *et al*., 2019). Chernozems and Kastanozems have prominent accumulation of secondary carbonates, while Phaeozems are generally slightly leached and decarbonated but still have high ($\geq$ 50 %) base saturation (BS) (IUSS Working Group WRB, 2015). These soils are the most dominant and fertile soil units in Hungary.

They have favorable attributes that include deep humus rich fertile surface layer, good water holding capacity, easily available nutrients, and good workability (Birkás *et al*., 2012).

Soils with clay accumulation: in the traditional genetic approach, these soils belong to brown forest soils. They are formed on the hills that the brown forest soils with clay illuviation are more common in the flat surfaces. In the modern HSCS, they are represented by the occurrence of the clay accumulation horizon that corresponds with WRB argic horizon. Hence, they match with the Luvisols of the WRB. In a condition where the acidification processes are intensive, the Alisols RSG may be the corresponding WRB unit (Michéli *et al*., 2019). Luvisols represented with subsurface clay accumulation with high (≥ 50%) BS while Alisols represented with intensively leached and acidified subsurface clay accumulation with low (< 50%) BS (IUSS Working Group WRB, 2015). In forested areas where high humification process may result in deep, high organic carbon containing surface horizons, they may correlate with the WRB Umbrisols (Michéli *et al*., 2019).

Sandy soils: The typical characteristics of having a weighted average texture class of sand or loamy sand to a depth of 1 m from the soil surface, or to a depth of a cemented or indurated layer, whichever is shallower, matching them with the WRB Arenosols RSG. They are weakly developed soil with low organic and inorganic colloids that resulted in low soil fertility (Michéli *et al*., 2019; IUSS Working Group WRB, 2015). In Hungary, Arenosols have developed on windblown sand deposited after the end of the last ice age and are extensive in certain parts of the country (Michéli *et al*., 2019).

Solonetz soils: These are salt affected soils characterized by the presence of high amount of adsorbed sodium and/or magnesium, and the strongly structured columnar subsurface horizon, correlate with WRB Natric horizon. Commonly found in the Hortobágy, the Körös Region and the Tisza Valley (Mezősi, 2017). Solonetz soils are the WRB equivalent for these soils. These soils are common in lowland areas with high evaporation rate.

Swelling clay soils: soils characterized by high activity clay content and shrinking and swelling properties due to alternating dry and wet conditions (Michéli *et al*., 2019). In the traditional HSCS, no separate unit defined for these soils, rather allocated in several different soil taxonomic units such as meadow, salt affected, parent material influenced, and alluvial main soil types (Fuchs, 2012). In the WRB system, these soils fall under Vertisols.

Meadow soils: are groundwater affected soils of lowland areas with redoximorphic features and black A horizon due to the high humidity and the organic matter in anaerobic environment. They

are typical for alluvial sediments, for example, to the calcareous sediments of the Danube and Hernád River, and the acidic sediment of the Tisza, Rába and Körös Rivers (Mezősi, 2017). Meadow soils showing reducing conditions within 50 cm from the soil surface, and evidence of the gleyic color patterns in more than 50 per cent of the matrix between 50–100 cm from the soil surface likely correlate with the WRB Gleysols. Other groundwater affected soils with redoximorphic features in more deeper soil depth may belong to other WRB RSG with Gleyic qualifier (Michéli *et al*., 2019).

Brown earths: They are typical members of the brown forest soils in the old classification system. They are characterized by subsurface horizon that shows light alteration in color and soil structure compared to the parent material. This definition corresponds them with the Cambisols reference group of WRB. However, the brown earths soils may fall in to WRB Calcisols, if they are having calcic horizon within 1 m depth of the soil (Michéli *et al*., 2019).

2.6.2. Major soil types of Ethiopia

Ethiopia is bestowed with diverse soil types due to diversity in climate, parent material and landform positions (Girmay *et al*., 2008). Numerous soil studies have been conducted in the country, mostly based on Provisional Soil Association Map of Ethiopia at 1:2 million scale (FAO, 1984) which identified 19 soil types, among which, Leptosols (17%); Nitisols (12%); Cambisols (11.6%). Regosols (10.9%); Vertisols (10%); Fluvisols (8%) and Luvisols (6%) are the most common RSGs. However, the map is not adequately supported by profile data and field surveys (Elias, 2016). To address the issue, Ethiopian Soils Information System (EthioSIS) collaborated with Ethiopia-Netherlands bilateral research project, "Capacity building for scaling up of evidence-based best practices for increased agricultural production in Ethiopia (CASCAPE) characterized, classified and mapped soils in 30 high agricultural potential highland woredas (districts) based on detailed profile data. The project identified 15 major RSG, among which, Nitisols (31%), Vertisols (27%); Leptosols (26%), Luvisols (11%), Planosols (2%), Regosols (2%), and Cambisols (0.9%) are the most prominent RSGs.

Nitisols: Nitisols are deep, well-drained, red tropical soils with diffuse horizon boundaries. They are characterized by a clay rich 'nitic' subsurface horizon that has polyhedric, blocky structure elements with shiny ped surfaces (IUSS Working Group WRB, 2015). Nitisols covers 30% of the land mass in CASCAPE survey districts (Elias, 2016). In Ethiopia, Nitisols predominantly occur in the humid south-western highlands (64%) and north-western highlands (21%). Although Nitisols in Ethiopia spread over a wide range of slope gradient, mostly they are found on the upper/middle slop position (Elias, 2016). In the undulating landscapes, Nitisols situated on upper

and middle slope positions intergrading with Vertisols in the lower position while in volcanic land scape Nitisols found on mid-slope position integrating with Andosols and Luvisols at higher slope positions and with Vertisols at the lower slope positions. Luvic Nitisols, Haplic Nitisols, and Mollic Nitisols are the most abundant sub-groups. Nitisols are derived from volcanic parent materials, such as basalt, trachyte, tuff, ignimbrites, etc. by strong weathering, but they are more fertile than other weathered tropical soils (De Wispelaere *et al.*, 2015).

Nitisols in Ethiopia are intensively utilized for agricultural cropping purpose mainly to produce wheat, teff, barley, and faba bean, thus very important for food production in the country. Most of the Nitisols in Ethiopia are formed on volcanic deposits and with continued volcanic activity. Surface accumulation of volcanic ash followed by incorporation of it constitute in the soil through biological activity and other types of pedoturbation result in a relatively high silt content, suggesting the silt/clay ratios of the WRB are not a suitable requirement for the definition of nitic horizons (De Wispelaere *et al*., 2005). Generally, Nitisols in Ethiopia have high SOM content, CEC, and BS. However, they are constrained by very high soil acidity, low level of available phosphorous, sulfur and exchangeable potassium. Moreover, due to the landform of occurrence, they are susceptible for water erosion (Elias, 2016).

Vertisols: Vertisols are heavy clay soils with a high proportion of swelling clay that can swell and shrink in response to a change in soil moisture. They are typically characterized by slickensides, polished, and grooved shiny surfaces, produced by one mass of soil sliding past another (IUSS Working Group WRB, 2015). Vertisols are one of the very common agricultural soils in Ethiopia accounting 27% of the landmass in CASCAPE districts (Leenaars, 2016). Vertisols are widely distributed throughout the country but largely found in the volcanic plateaus and the colluvial slopes and foothills of the north-central highlands. They are dominantly formed on alluvial/colluvial deposits from upslope-weathered rocks of volcanic origin. The majority of Vertisols (80%) occur on gently sloping to moderately steep (5-30%) gradient slopes with impeded drainage. Vertisols associate with Cambisols and Luvisols in better drained landscape position, while they intergrade with Planosols (stagnic) and with Gleysols (gleyic) on least well-drained central parts of the plains and low-lying areas. In more arid areas, Vertisols may intergrade with Calcisols and Gypsisols and on wetter and humid climates, they intergrade with Phaeozems and Chernozems. The most common subdivisions of Vertisol according to IUSS Working Group WRB (2006) are Haplic, Calcic, Gleyic, and Grumic Vertisols (Elias, 2016).

Vertisols are one of the most common and intensively utilized agricultural soils in Ethiopia. Generally, the soils have high productive potential with high CEC, BS, moisture retention

capacity, and good soil structure. However, Vertisols in Ethiopia tend to have low available plant nutrients, organic matter, and poor in workability (Giday, 2015).

Leptosols and Regosols: Leptosols are very shallow soils over continuous rock and soils that are extremely gravely and/or stony soils. Regosols are weakly developed soils in unconsolidated mineral materials that are not very thin or very rich in gravels (Leptosols) and coarse textured materials (Arenosols) do not have materials with fluvic properties (Fluvisols) and are lacking a Mollic or Umbric horizon (IUSS Working Group WRB, 2015). Leptosols are one of the dominant soils accounting 30 and 26% of the total landmass of Ethiopia and in CASCAPE woredas, respectively. They are typical of areas of steepy slope (30-45%), dry climate, and geologically too young (e.g., in the case of recent lava flows). They are commonly found in the north eastern and central highlands including north Shewa, north Wollo, east Gondar, and in many parts of Tigray and the Hararghe plateau (Elias, 2016). Regosols are extensively found in eroding lands, particularly in arid and semi-arid areas and in mountainous terrain (IUSS Working Group WRB, 2006). Regosols accounted 1% of the land mass in CASCAPE districts and most abundantly (about 97%) found in the south-eastern highlands where the slope ranges between 15 to 45%. Leptosols and Regosols in Ethiopia are exclusively formed on volcanic parental materials. Hepalic Leptosols and Leptic Regosols are the dominant subunit (Elias, 2016). Low soil organic matter and acute deficiency of nutrients such as N, S, K, and Zn are the important fertility issues of these soils. Furthermore, limited rooting depth is another challenge that reduce the agricultural potential of these soils (Giday, 2015).

Luvisols*:* Luvisols are among the prominent soil types in Ethiopia accounting for about 11% of the total landmass of the CASCAPE districts, typically found on north-central and south-south-western highlands. The geomorphic environment of Luvisols in Ethiopia is similar with Nitisols, dominate the undulating to rolling plateaus, high relief hills and dissected side. However, Luvisols tend to occupy the side slopes of volcanoes of relatively young age occurring at higher elevations than the Nitisols occupying the adjacent plateaus of relative old age. The majority of them are found on sloping (5-10%) to moderately steep (15-30%) gradients. In the higher position they co-exist with Nitisols and in the lower sloping position with Vertisols. They are exclusively formed from *in-situ* weathered volcanic rocks such as basalt. Haplic Luvisols are the dominant unit accounted 60% of the soil profiles investigated by CASCAPE surveyed districts (Leenaars, 2016; Elias, 2016).

Luvisols of Ethiopia are among the important agricultural soils in the country. Their fertility mainly related to attributes including adequate rooting depth, stable structures, and good water

holding capacity, and high CEC and base cations. Nonetheless very low organic matter content and nutrient deficiency coupled with strongly acidic soil reaction related to long-term use of DAP, are the main fertility challenges (Geta *et al*., 2013; Negassa and Gebrekidan, 2003).

Cambisols*:* Cambisols are young soils with limited pedogenetic changes. They undergone various pedological alteration which is enough to distinguish them from other shallow soils, such as Leptosols, Regosols but not enough for the development of horizon that is needed for the classification of other major groups (IUSS Working Group WRB, 2015). Cambisols mostly occur in higher altitudes and on steep slope position where conditions restricted the pedological process and development of the soil. Cambisols occur in a wide geomorphological environment, therefore, integrate with almost every RSGs. Cambisols are extensively found in Tigray and the Hararghe highlands. They are form on a mixture of various parent materials including volcanic, calcareous limestone and alluvial and colluvial deposits. Haplic Cambisols are the most abundant subgroup in Ethiopia (Elias, 2016).

Cambisols have favorable soil properties such as good structure, moderate water holding capacity, nearly neutral pH, moderately adequate levels of P, high CEC, and base cations content. Like other RSGs of Ethiopia, low levels of organic matter and total N are a serious fertility issue of Cambisols (Elias, 2016).

2.7. Overview of related soil biodiversity studies in Hungary and Ethiopia

The chemical, mineralogical, physical, hydraulic of major soils of Hungary and their classification have been well documented (Gangwar *et al*., 2018). However, the biological properties and the effect of soil and LUT on the biological component of the soil were not widely investigated (Mucsi *et al*., 2017). Few studies focused on microbial activity and properties of salt affected soils (e.g. Abdoussalam *et al*., 2005; Gangwar *et al*., 2018), effect of temperature and organic matter on soil respiration in deciduous oak forest (Kotroczó *et al*., 2014), microbial properties of Hungarian sandy soils under different management practices (Demeter *et al*., 2018), influence of erosion on soil biodiversity (Simon *et al*., 2011), soil bacterial diversity (Knáb *et al*., 2018), effect of soil physical state (Birkás *et al*., 2010) and tillage (Birkás *et al*., 2012; Dekemati *et al*., 2019) on earthworms, the response of springtails and mites to simulated repeated drought events of different magnitudes in a Hungarian semi-arid sand steppe (Flórián *et al*., 2019), extreme effect of drought in composition of soil bacterial community and decomposition of plant tissue (Tóth *et al*., 2017), abundance of soil bacterial communities from juvenile maize plants of a long-term monoculture

and a natural grassland (Ujvári *et al*. 2020), earthworm assemblages in urban habitat across geographical regions (Tóth *et al*., 2020) were reported.

Several studies have described and characterized the chemical and physical properties (e.g., Yitbarek *et al*., 2016; Deressa *et al*., 2018) and the influence of land use change on the physicochemical properties (Tufa *et al*., 2019; Lulu *et al*., 2019) of major soil groups in Ethiopia. Yet, the biological properties have rarely been studied and characterized. Very few studies are available on the effect of soil and LUT on soil biological properties e.g., effects of LUT and soil properties on arbuscular mycorrhizal fungi (Belay *et al*., 2013) and symbiotic bacteria (Aserse *et al*., 2013), soil microbial biomass and soil respiration between natural and adjacent plantation forest in Munessa Forest (Yohannes, 2017), land use impacts on physicochemical and microbial soil properties (Aredehey *et al*., 2019), PGPB from sugarcane (*Saccharum officinarum* L.) rhizosphere (Feredegn *et al*., 2015), soil microbial (bacteria and fungi) diversity and community composition across five land use systems in the highland of Ethiopia (Delelegn *et al*., 2018), soil microbial communities in original forest soils and adjacent degraded land in northwest Ethiopian highlands (Abebe *et al*., 2020).

3. MATERIALS AND METHODS

This chapter provides details of the study areas, sampling designs, earthworm extraction and laboratory procedures for soil and statistical analyses.

3.1. Site description

In total, thirteen sampling sites in Hungary and four in Ethiopia were investigated. The sites were chosen because of the availability of legacy data. The sites have been serving as experimental fields to conduct numerous researches for long period of time. Moreover, the sites represent some of the dominant RSGs of Hungary and Ethiopia, which was the main focus of this study.

Hungary

The study was carried out on experimental farms (JM1, JM2, JM3) of Hungarian University of Agriculture and Life Sciences (former Szent István University) at Józsefmajor nearby Hatvan (N 47° 40'5", E 19° 40′ 11"), nearby Hort city (HOGR, HOAR) (N 47º 4'36.90", E 19º 48'53.04"; N 47º41' 42.03", E 19º 48'50.46"), Verpelét (VERP) (N 47°52'8.85", E 20°11'59.61") in Heves County; Gödöllő hill (GUF, GBG) and Szárítópuszta (SZP1, SZP2) in Gödöllő town (N 47°35' 47.65", E 19° 21' 18.54"), Pest County; Szárhalmi forest (SZHE) (N 47° 41' 41", E 16°50' 31"), Károly-magaslat (KAMG) (N 47° 39' 49.14", E 16° 33' 41.10") in Győr-Moson-Sopron County; Csobánc (CSOB) (N 46° 52' 18.50"; E 17° 30' 16.35") in Veszprém County (Figure 1).

The experimental farm of Józsefmajor, Hort, and Verpelét are part of 'North Plain Alluvial Fan' which is a small geographical area of the Northern Hungarian Mountain. The average annual temperature ranges 10–11ºC and the mean rainfall is between 550–600 mm. Most soils of the region are formed in weathered, clayey sediment mixed or covered with loess, or in loess and are underlain with Miocene lake sediments (Pannonian sand and clay). Luvisols and Chernozems are the dominant soil types in the area. The elevation of the region varies between 128 and 350 m. The mean annual precipitation ranges from 580–610 mm and the mean annual temperature is 9.5–10ºC (Dövényi *et al*., 2008).

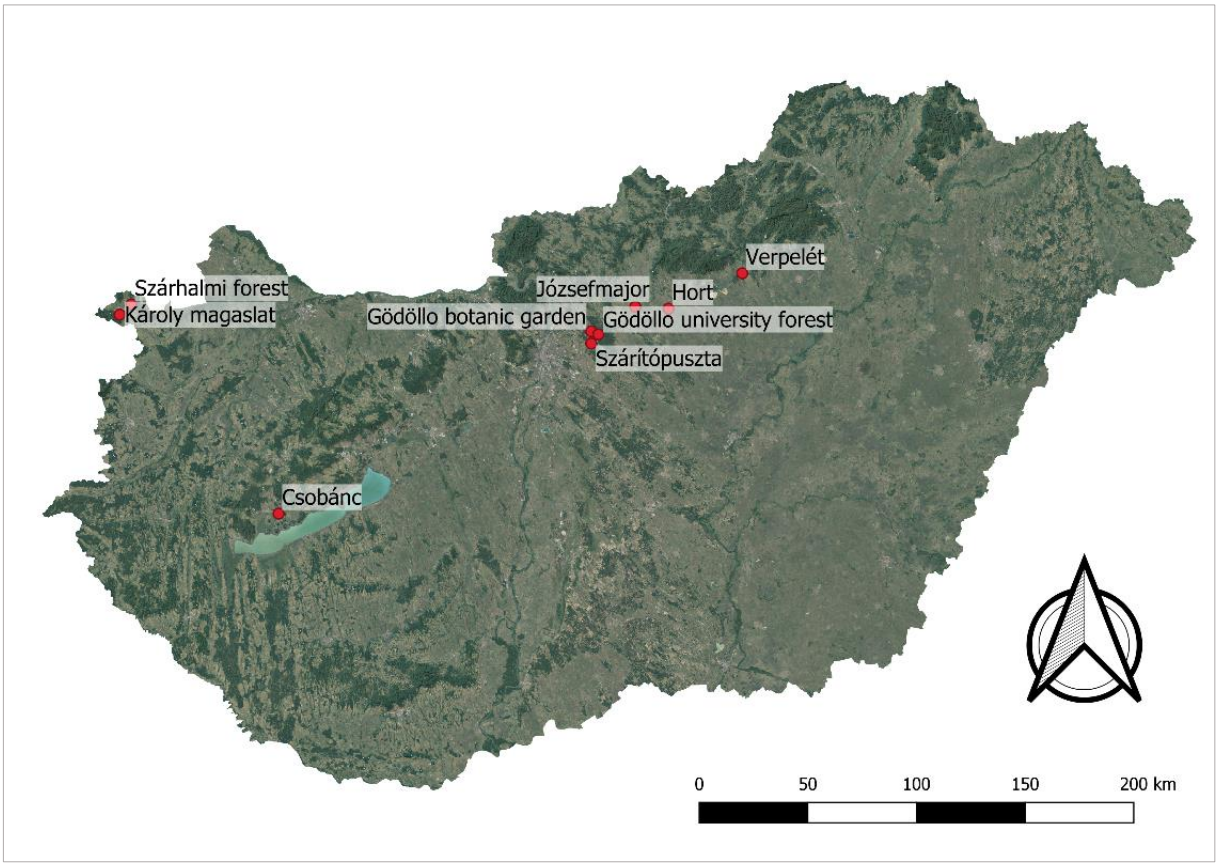


Figure 1. Location map showing study sites in Hungary

The Gödöllő sites (GUF, GBG, SZP1, SZP2) belong to the Gödöllő-Monori hilly region which is part of Northern Hungarian Mountain. The most common RSGs in the region are Luvisols, Cambisols, Arenosols, and Chernozems; formed on loess and alluvial sediments formed during the Pleistocene and Holocene epoch. The mean annual temperature ranges from 9.5–10^{o}C and the annual precipitation is about 600 mm (Dövényi *et al*., 2008).

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

According to USDA Soil Taxonomy (USDA 1993), the soil moisture regime at the study sites is ustic and the soil temperature regime is mesic.

LUTs and soil management systems in the sampling sites

The study sites encompassed three LUTs, i.e., forest, grassland, and arable land. The Gödöllő forests are dominated by oak trees (*Quercus cerris* and *Quercus robur*) grown more than 50 years while forests of Szárhalmi and Károly-magaslat are mainly composed of *Quercus petrea, Fagus silvatica, Larix decidua, Picea abiessites* and *Carpinus spp.*

The predominant grass species in Hort site is *Elyumus repens* and has not been cultivated for 6 years. Szárítópuszta grassland is predominated by *Echinochloa crus, Echinochloa galli, Setaria pumila, Chenopodium album, Fallopia convolvulus,* and left undisturbed more than 20 years. The grassy vegetation of Józsefmajor and Csobánc were unmanaged pasturelands.

The arable sites of Józsefmajor were ploughed with conventional disc (10-14 cm depth) and sown with winter oat (*Avena sativa L.*). The soils were treated with 100 kg ha^{-1} CAN (NH_4NO_3 + $CaMg(CO_3)_2$) fertilizer. The Hort arable soils were subjected to intensive tillage by harrowing with heavy disc and 150 kg N ha^{-1} was applied in the form of 34% ammonium nitrate. The Szárítópuszta arable site was tilled with cultivator and complex NPK fertilizer (15/15/15), 100 kg^{-1} CAN (NH_4NO_3 + $CaMg(CO_3)_2$) fertilizer was applied. During the sampling, the cultivated crop on Hort was oilseed rape (*Brassica napus*) and that of Szárítópuszta was winter oat. The Verpelét site was ploughed conventionally, and it was cultivated with wheat in 2017 and formerly with alfalfa for 5 years.

Ethiopia

The study was conducted in Laelay Maichew and Atsbi Wenberta districts. Laelay Maichew district is geographically located at longitude 13° 55' 53" E and latitude 38° 12' 19" N in the central part of Tigray Regional State: one of the nine Regional States of Ethiopia (Figure 2). The district altitude varies between 1842 and 2250 m. It is agro-ecologically classified in the semiarid region characterized by a short rainy period. The rainfall pattern is typically unimodal with the main wet season (kiremt) extending from July to September and the average annual rainfall ranges between 550–750 mm. Similarly, the mean annual minimum and maximum temperatures are 11.7^0C and 26.1^0C, respectively (Kahsay and Mulugeta, 2014). The geology of the area is characterized by rocks of varied composition ranging in the age Precambrian to Quaternary. The Precambrian basement rocks comprise weakly metamorphosed acidic to basic lava and pyroclastics, volcanoclasic, detrital and chemically precipitated marine sediments. Intrusive, Paleozoic-Mesozoic sedimentary strata, tertiary volcanic and Quaternary deposits are also found (Tadesse, 1998). The main RSGs are Cambisols on undulating plains and rolling landforms; Leptosols on hilly and steep to very steep lands, Vertisols are found on the flat plateau plains, Luvisols

predominantly found on sloping (5-10%) to moderately steep (15-30%) gradients together with Nitisols on the sloping positions and with Vertisols on the lower slope positions (Brhane and Mekonen, 2009). The land use is predominantly shrubland (44%) followed by cropland (33%) and settlement area (8%). The farming system is crop farming mixed with livestock husbandry. Teff cultivation (*Eragrostis tef (Zuccagni) Trotter)* accounts for most arable lands and followed by wheat (*Triticum aestivum L*) crop (Brhane and Mekonen, 2009).

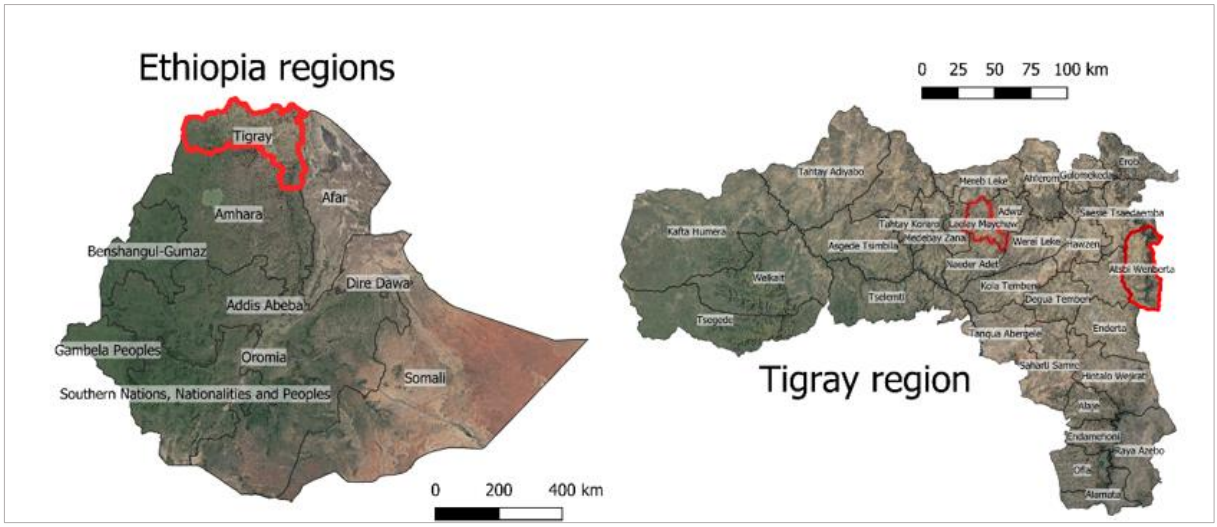


Figure 2. Location map showing Tigray region within Ethiopia and Laelay Maichew and Atsbi Wenberta districts within Tigray region

Atsbi Wenberta district is situated in the eastern part of Tigray Regional state. Geographically, it is bounded between 39° 30'-39° 45' E and 13° 30'- 13° 45' N. The elevation of the district varies from 918 to 3069 m. 75% of the district is upper highlands (2600 m or above) and only 25% is found in midlands (between 1500 and 2600 m) and lowlands (below 1500 m) (Gebremedhin, 2004). The district falls in sub-tropical agro-climatic zone and has an average daily temperature between 15°C and 30°C and the mean annual precipitation rate is about 529 mm. The district is drought prone with intense and short duration rainfall, hence, the soils are susceptible to erosion due to high run-off (Gebremedhin, 2004). The geology of the district is dominated by Adigrat sandstone lithology (Bekele *et al*., 2012). Lithic Leptosols is a predominant soil type in the area (Gebremedhin, 2004).

Agricultural management systems of the sampling sites

All the four sites were arable land ploughed traditionally by oxen for more than 50 years. Prior to the soil sampling the fields in Laelay Maichew and Atsbi Wenberta were under cultivation of teff

and wheat, respectively. Teff, a warm-season annual cereal, is a major indigenous staple crop in Ethiopia. The fields were treated by N: 32.5 kg ha^{-1}, P_2O_5: 18.8 kg ha^{-1}, K_2O: 3.4 kg ha^{-1}.

3.2. Criteria used to select the studied RSGs

In Hungary, the following seven RSGs were chosen; Chernozems, Kastanozems, and Phaeozems (Steppe soils), Arenosols (Sandy soils), Luvisols and Alisols (soils with clay accumulation), and Vertisols (Swelling clay soils). These RSGs were chosen because: i) they represent different geographical environments (climate, geographic and genetic) of the country, ii) their dominancy in terms of geographical extent, and in the case of steppe soils, due to their great agricultural importance (Michéli *et al*., 2019).

Among the existing countrywide soil maps in Ethiopia, the recent and most prominent one is the CASCAPE soil map. The map was developed based on an extensive soil survey conducted in 30 high agricultural potential districts across four major regions of the country. The districts are "representative" of the major agro-ecologies, farming systems and crop belts in the Ethiopian highlands. Based on that, Nitisols (31%), Vertisols (27%); Leptosols (26%), Luvisols (11%), Planosols (2%), Regosols (2%), and Cambisols (0.9%) are the most prominent RSGs. For this study, Nitisols, Vertisols, Luvisols, and Cambisols have been selected due to their geographical extent (most prevalent soils) and high agricultural potential (Elias, 2016).

3.3. Soil profile description, characterization, and classification

Soil profiles were described and characterized according to FAO Guidelines (2006) and classified based on IUSS Working Group WRB (2015). Soil profiles in Hungary was previously described and characterized. In this study, only soil classification was performed. Based on that, the reference soil groups (RSG) of Józsefmajor and Hort city are Chernozems; Gödöllő hill sites have Luvisols; soils at Szárítópuszta sites are Phaeozems and Arenosols; Sopron sites has Alisols and Kastanozems; soils of the Csobánc and Verpelét are Phaeozems and Vertisols, respectively.

The fieldwork work in Ethiopia was carried out on 21st and 22nd November 2017. It included an exploratory soil survey, site and profile description, and sample collection. During the exploratory soil survey (reconnaissance) extensive augering was conducted to describe and to identify the existing soils types and their boundaries. Four-point locations for soil observation (three in Laelay Maichew and one in Atsbi Wenberta districts) were selected and georeferenced for the detailed studies. According to the IUSS Working Group WRB (2015), Laelay Maichew soils are Luvisol, Cambisol, and Vertisol while the Atsbi Wenberta is Nitisol. Details of site and soil profile description found in Appendix I and II for sites in Hungary and Ethiopia, respectively.

3.4. Soil sampling

Hungary

The first soil sampling was carried out on seven designated soil profiles i.e., three at Józsefmajor (JM1, JM2, JM3), two at Gödöllő hill (GUF, GBG) and two at Szárítópuszta (SZP1, SZP2) sites in October 2017 (Figure 3).

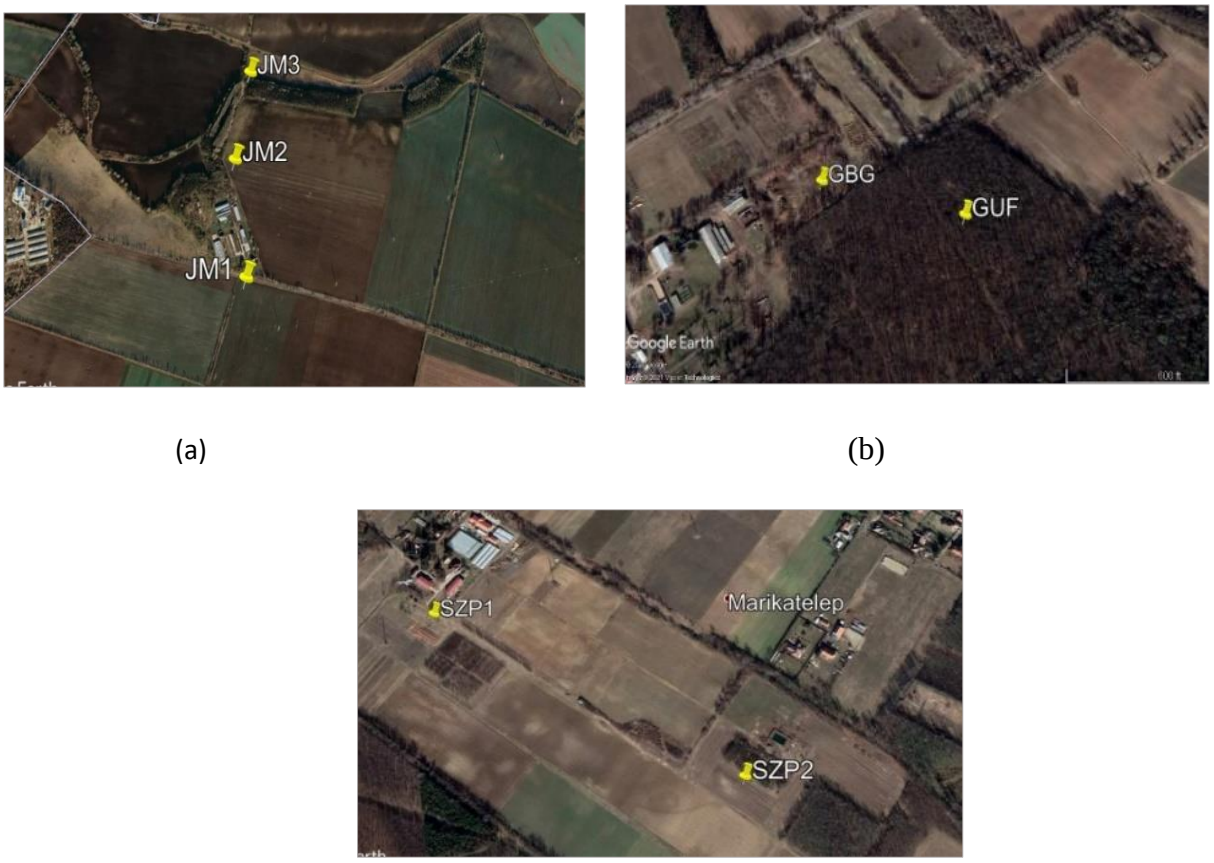


Figure 3. Google Earth maps showing LUTs and soil profile locations of a) Józsefmajor b) Gödöllő hill c) Szárítópuszta sites

The second soil sampling was performed in November 2018 from the following sites: Gödöllő hill, Szárítópuszta, Szárhalmi forest (SZHE) and Károly-magaslat (KAMG) in Sopron city, Csobánc (CSOB), and Hort city. (HOGR, HOAR). Furthermore, soil samples from Verpelét (VERP) were taken in 2021 (Figure 4).

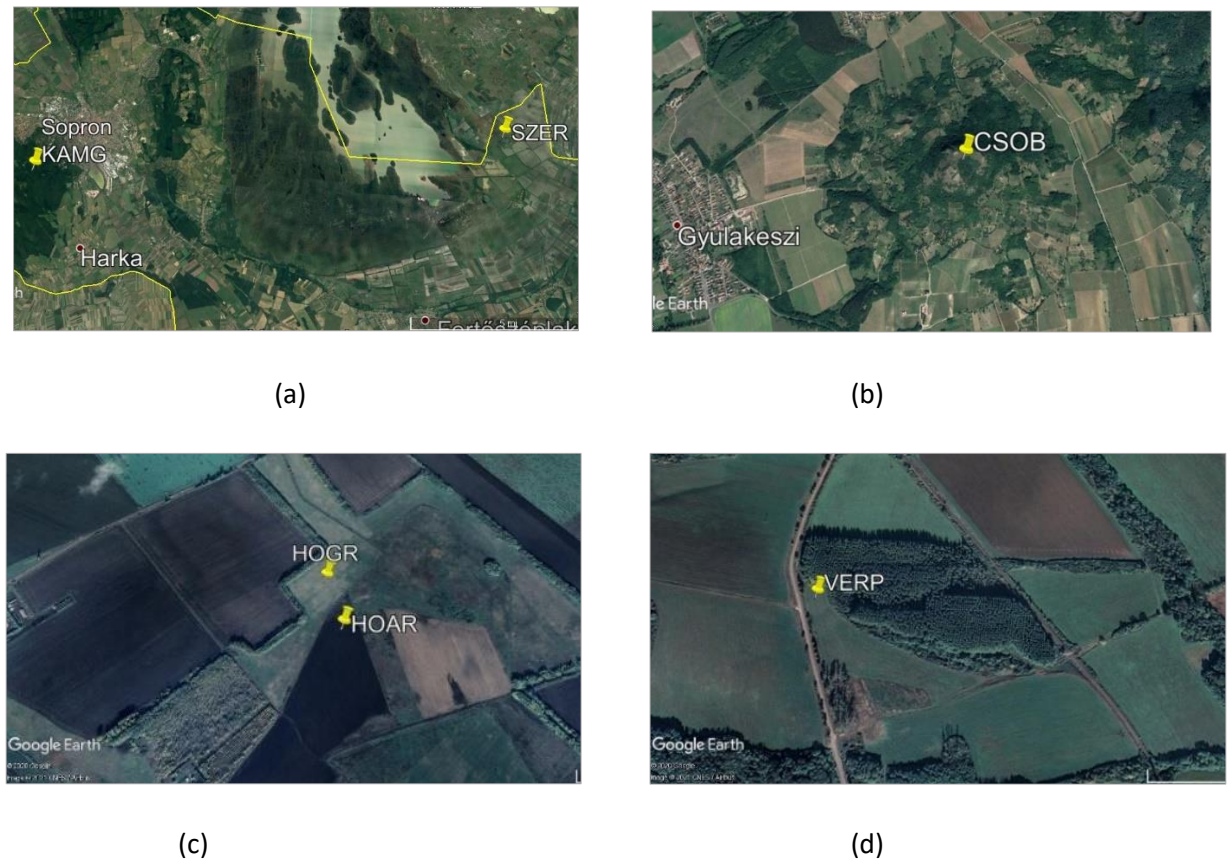


Figure 4. Google Earth maps showing LUTs and soil profile locations of a) Szárhalmi and Károly-magaslat b) Csobánc c) Hort d) Verpelét sites

Soil samples from Józsefmajor, Gödöllő hill, Szárítópuszta, Hort, and Verpelét sites were taken from the upper 25 cm of the soil. However, soil samples from Szárhalmi forest, Csobánc, and Károly-magaslat sites were collected along the two depths of the soil (0–10 cm and 10–25 cm). Samples were collected on 1m × 1m plots, three meters away from the main soil profile, in three different directions as shown in Figure 5. From each plot, one bulk soil sample, roughly measured 1 kg and three undisturbed soil cores (total 63) (only during the first sampling) for bulk density were taken using spade and volumetric core, respectively. Samples from each soil profile and/or the same depth (1–10 or 10–25 for Szárhalmi forest, Csobánc, and Károly-magaslat sites) compiled together and mixed thoroughly, divided into two subsamples, one portion for SMR and the other for physicochemical analyses. Soil samples for physicochemical analyses were saved under 2 mm mesh and stored at room temperature while soil samples for SMR were stored in refrigerator at 4°C.

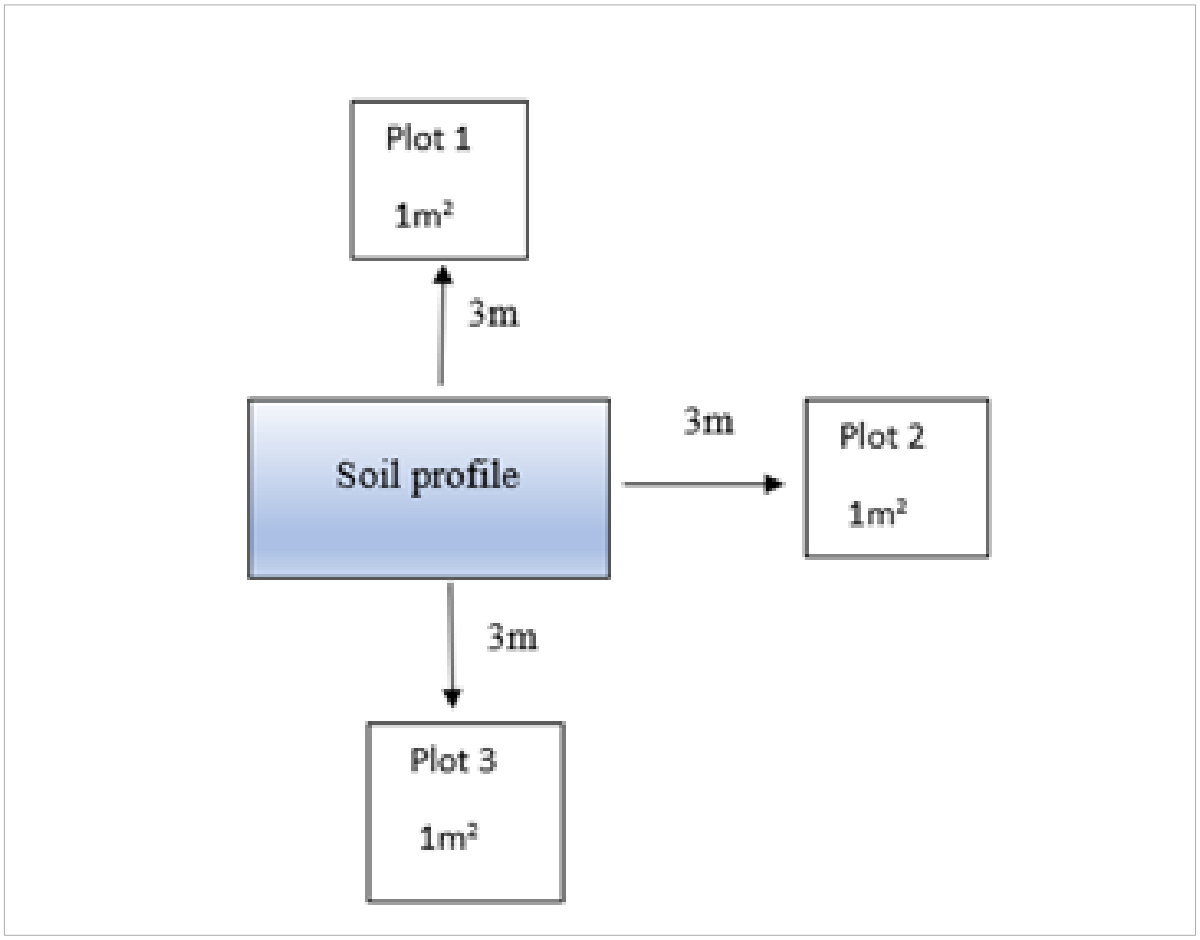


Figure 5. Soil sampling scheme used in Hungary.

Ethiopia

Soil sampling was carried out on January 2–4, 2019 from four agricultural fields (three from Laelay Maichew and one from Atsbi Wenberta (Figure 6).

(a) (b)

Figure 6. Google Earth maps showing soil profile locations of a) Laelay Maichew b) Atsbi Wenberta sites

At each site, eight points around the main soil profile in a 10 m radius were designated as sampling points (Figure 7). Soil samples from a depth of 0–25 cm, were collected from these points, compiled, and mixed thoroughly to make a composite sample. From the composite sample, three subsamples were taken for: a) physiochemical, b) SMR, c) soil bacterial genomic analyses. Soil samples for microbial respiration and DNA analyses were sieved on-site through 2 mm sieve to remove stones, roots, macrofauna, and litter materials, and transported in ice box then stored in refrigerator (4°C) until analysis. During soil sampling and soil processing, considerable care was taken to avoid contamination between the samples by using gloves and cleaning all equipment with 70% ethanol. Soils for physicochemical analyses were air dried and sieved through 2 mm mesh and stored in room temperature until the analyses. Except for the moisture content, all analyses for the soils from Ethiopia were conducted in the laboratory of Institute of Environmental Sciences, Hungarian University of Agriculture and Life Sciences, Gödöllő, Hungary, three weeks after the soil sampling took place. The summary of the sites, soil sampling and parameters determined is presented in Table 1.

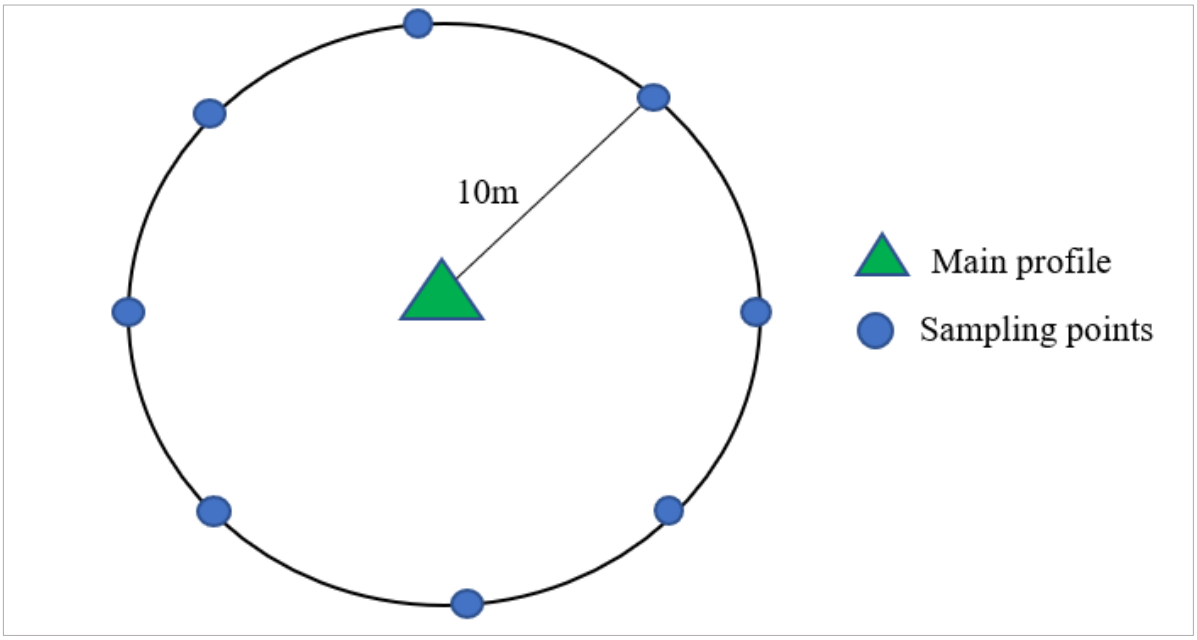


Figure 7. Sampling scheme used in Ethiopia

Table1. Summary of sites, soil sampling, and determined parameters

Site	**Abbreviation**	**LUT**	**RSG**	**Sampling depth**	**Year of sampling**	**Physicochemical parameters**	**Biological parameters**
Szárítópuszta 1	SZP1	Grassland	Phaeozem	0-25cm	2017/2018	SOM, $CaCO_3$, pH, available NPK, E_4/E_6, MC, BD, base cations (K, Mg, Ca, Na), CEC, and BS, texture	Earthworms (EW) (biomass, abundance, species richness) Soil microbial respiration (SMR)
Szárítópuszta 2	SZP2	Arable	Arenosol				
Gödöllő university forest	GUF	Forest	Luvisol				
Gödöllő botanical garden	GBG	Forest	Luvisol				
Józsefmajor 1	JM1	Arable	Chernozem	0-25cm	2017	SOM, $CaCO_3$, pH, available NPK, E_4/E_6, MC, BD, and base cations, texture	EW SMR
Józsefmajor 2	JM2	Arable	Chernozem				
Józsefmajor 3	JM3	Grassland	Chernozem				
Szárhalom forest	SZHE	Forest	Kastanozem	0-10cm 10-25 cm	2018	SOM, $CaCO_3$, pH, available NPK, E_4/E_6, base cations CEC, and BS, texture	SMR
Károly-magaslat	KAMG	Forest	Alisol				
Csobánc	CHOB	Grassland	Phaeozem				
Verpelét	VERP	Arable	Vertisol	0-25cm	2020	SOM, $CaCO_3$, pH, MC, base cations, CEC, and BS	SMR, EW
Hort 1	HOGR	Grassland	Chernozem	0-25cm	2018	SOM, CaCO3, pH, MC, available NPK, E4/E6, base cations, CEC, and BS	SMR
Hort 2	HOAR	Arable	Chernozem				
Laelay Maichew 1	LMH-1	Arable	Luvisol	0-25 cm	2019	SOM, CaCO3, pH, MC, available NPK, E4/E6, base cations, CEC, and BS	SMR Bacterial genome
Laelay Maichew 2	LMH-2	Arable	Cambisol				
Laelay Maichew 10	LMH-10	Arable	Vertisol				
Atsbi-Wenberta 3	ATS-3	Arable	Nitisol				

Note: *BD was only measured for soil samples collected in 2017; CEC and BS were measured for 2018 and 2019 soil samples; EW not collected from SZP, GUF, and GBG in 2018*

Abbreviations: soil organic matter (SOM), soil moisture content (MC), cation exchange capacity (CEC), base saturation (BS), bulk density (BD), soil microbial respiration (SMR)

3.5. Laboratory soil measurements and earthworm extraction

3.5.1. Physicochemical soil analyses

All laboratory analyses were performed in triplicate for each soil samples. Soil pH was measured potentiometrically on soil suspended in a solution of deionized water and 1M KCl in 1:2.5 ratio (w/v) (Buzás, 1988). Bulk density and soil moisture content were determined by gravimetric method at 105°C for 24 h (Buzás, 1993). Available nitrogen (NH_4^+-N and NO_3^--N) was measured using Parnas-Wagner Apparatus (Egnér *et al*., 1969). Available potassium and phosphorus were estimated based on ammonium-lactate solution method (AL method) using flame photometer and UV-VIS spectrophotometer, respectively (Egnér *et al*., 1960). For soil organic matter analysis, soil samples were grinded, passed through 0.2 mm mesh, 0.200–0.2020 g of soil was measured, and its organic carbon content was measured using Walkley-Black method (Walkley and Black, 1934). $CaCO_3$ content was determined using Scheibler calcimeter (Buzás, 1988). Available Ca^{2+}, Mg^{2+}, and Na^+ were extracted in 1N KCl, determined by EDTA titration, and measured by AAS at wave lengths of 422.7 and 285.2 nm, respectively (Egnér *et al*., 1960). Cation exchange capacity and exchangeable basic cations (Ca^{2+}, Mg^{2+}, K^+, and Na^+) were extracted following the Mehlich 3 extraction (Mehlich, 1953), and the base cations were measured using ICP-spectrometer. The humus quality measured by E_4/E_6 (ratio of the absorbances alkali extracted organic matter at 465 nm and at 665 nm) was determined using spectrometer (Page *et al*., 1982).

3.5.2. Bacterial genomic analyses

DNA isolation and purification

For extracting the total DNA, the method of Högfors-Rönnholm *et al.* (2018) was carried out modified with sonication on ice for one-minute sonication and 1-minute brake the slurry in 50 ml falcon tubes for one minute in three rounds after vortexing. DNA was extracted from the collected supernatant using Quick-DNA Soil Microbe Miniprep Kit (Zymo Research, USA) according to the manufacturer's instruction. DNA was visualized by gel electrophoresis. Furthermore, the quality and the integrity of isolated DNA was determined by Nanodrop spectrophotometer ND 2000 (Nano-Drop Technologies, Wilmington, DE, USA).

16S rDNA amplicon sequencing and data handling

To assess the bacterial community composition of the soils sample precisely, Illumina 16S rDNA amplicon sequencing was carried out. The variable V3 and V4 region of the 16S rDNA was amplified by using the primers recommended by Klindworth *et al.* (2013), 16S amplicon PCR forward (5′-**TCGT CGGCAGCGTCAGATGTG TATAAGAGACAG**CCTA CGGGNGGCWGCAG-3′) named 16S Amplicon PCR Forward Primer-S-D-Bact-0341-b-S-17-N

and reverse (5′-**GTCT CGTGGGCT CGGAGATGTGTATAAGAGAC AG**GACTACHVGGGTATCTAATCC-3′), named 16S Amplicon PCR Reverse Primer-S-D-Bact-0785-a-A-21-N primers with Illumina adapter overhanging nucleotide sequences written in bold (Klindworth *et al.*, 2013). PCR reaction mixture in a final volume of 50 µl that contained 12.5 ng of DNA, 0.2 µM of each Illumina 16S primers and 12.5 µl of 2X KAPA HiFi Environ Sci Pollut Res HotStart Ready Mix (KAPABiosystems, London, United Kingdom). The temperature profile used was an initial denaturation for 3 min at 95°C, followed by 25 cycles of denaturation for 0.5 min at 95°C, annealing for 0.5 min at 55°C and elongation for 0.5 min at 72°C. The last step was a final extension for 5 min at 72°C. All amplifications were carried out in a ProFlex PCR System (Applied Biosystems by Life Technologies, USA). Amplicons were analyzed under UV light after electrophoresis in 1% (w/v) agarose gel stained with EtBr. Paired-end fragment reads were generated on an Illumina MiSeq sequencer using MiSeq Reagent Kit v3 (600-cycle). Read numbers were the following: 77065 for LMH-1 for sample, 60265 for LMH-10 and 67937 for LMH-2 and 58532 for ATS-3 sample (BF). Primary data analysis (base-calling) was carried out with Bbcl2fastq^ software (v2.17.1.14, Illumina). Reads were quality and length trimmed in CLC Genomics Workbench Tool 9.5.1 using an error probability of 0.05 (Q13) and a minimum length of 50 nucleotides as a threshold. Trimmed sequences were processed using mothur v1.35 (Schloss et al., 2009) as recommended by the MiSeq SOP page (http://www.mothur.org/wiki/MiSeq_SOP downloaded at 22/06/2019) (Kozich *et al.*, 2013). Sequences were assorted based on the alignment using SILVA 132 SSURef NR99 database (Quast *et al.*, 2013). Chimera detection was performed with mothur's uchime command (Edgar *et al.*, 2011), and 'split.abund' command was also used to remove singleton reads according to (Kunin *et al.*, 2010). After all quality control, 38132 reads/sample (400 bp/read) were taxonomically investigated. Taxonomic assignments were made against SILVA release 132 applying a minimum bootstrap confidence score of 80%. Operational taxonomic units (OTUs) were assigned at 97% similarity threshold level as suggested by Tindall *et al.* (2010) for prokaryotic species delineation. Raw sequence reads were deposited in NCBI SRA under BioProject ID SAMN14390016, SAMN14390017, SAMN14390018, SAMN14390019.

3.5.3. Soil Microbial respiration analyses

The analysis of soil microbial respiration (SMR) followed ISO 16072:2002(E) and Cheng *et al*. (2013) guideline with minor modification. Approximately 50 g fresh soil was placed in airtight jar and 10ml deionized water was added to adjust moisture content. A conical containing 10 ml 1.0 M NaOH was placed in the same jar and the samples were incubated for 10 days in dark at room temperature (22°C) (Figure 8). After 10 days, the conical was removed and 1 ml $BaCl_2$ was added in the NaOH solution to precipitate trapped CO_2. Two or three drops of phenolphthalein was added

(it turns the solution into pink). Then, the solution was titrated against 0.5M HCl till it become colorless. The determination was carried out in triplicates. Controls (triplicate flasks without soil) were also prepared.

Figure 8. Soil samples in incubation jar (Photo by Tsedekech, 2018)

3.5.4. Earthworm extraction and measurements (abundance, biomass, and species richness)

The extraction of earthworms was done by using hand sorting method as described by ISO 23611-1 (2006) guideline. According to the pattern shown in Figure 3, from each $1m^2$ plot, 25 × 25 × 25 cm soil blocks were taken using spade. The excavated soil was spread on the plastic sheet and earthworms were searched cautiously. Then the collected earthworms were placed in plastic bottles containing 70% ethanol. Later in laboratory, the earthworms were rinsed with tap water to remove the adhering soil particles from their body and transferred to 4% formalin for fixation and later preserved with 70% ethanol for species identification. To come up with the total abundance of earthworms, first, the number of worms were counted and expressed as individuals per sample. Second, the number earthworms in each sample were multiply by a factor (16) in order to achieve

the number of worms per square meter and the average was taken. The total biomass (g/m^2) was also estimated. The species richness was performed using identification key found in the guideline of Csuzdi and Zicsi (2003).

3.6. Statistical analyses

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

4. RESULTS AND DISCUSSIONS

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

This section presents the results of soil samples taken from Józsefmajor (JM1, JM2, JM3), Szárítópuszta (SZP1, SZP2), and Gödöllő (GUF, GBG) sites in 2017. Soils were grouped into mollic (Chernozems and Phaeozems) and non-mollic RSGs (Luvisols and Arenosols) based on the presence/absence of mollic diagnostic horizon. Physicochemical properties, SMR, and earthworm (abundance, biomass, and species richness) patterns were compared between these soil diagnostic categories and LUTs within each category.

4.1.1. Soil physicochemical properties

SOM ranged from 1.84% to 3.90% and from 0.98%, to 3.66%, and BD from 1.27 g cm^{-3} to 1.57 g cm^{-3} and from 1.11 g cm^{-3} to 1.51 g cm^{-3} in mollic and non-mollic soils, respectively. The pH-H_2O values ranged from 4.07 in GBG to 8.47 in SZP2. Both MC and the amount of exchangeable Mg^{2+} were highest in JM3 (28.58%, 39.67 mg kg^{-1}) and lowest in SZP2 (9.43%, 12.17 mg kg^{-1}). The mean available Ca^{2+} content varied from 687.67 mg kg^{-1} to 1588.00 mg kg^{-1} and from 268 mg kg^{-1} to 701 mg kg^{-1}, that of available Na^{+} from 6.78 mg kg^{-1} to 13.80 mg kg^{-1} and from 4.59 mg kg^{-1} to 6.55 mg kg^{-1} in mollic and non-mollic soils, respectively. $CaCO_3$ was only present at JM3, SZP1, and SZP2 sites, and significantly higher in SZP2. P_2O_5 was highest in JM3 and lowest in GUF. While K_2O was highest in JM3 and lowest in SZP2, NO_3^--N was highest in GBG and lowest in JM3. E_4/E_6 was highest in all non-mollic soils compared to mollic soils. NH_4^+-N ranged from 1.04 mg kg^{-1} to 5.09 mg kg^{-1} and from 3.3 mg kg^{-1} to 6.59 mg kg^{-1} in mollic and non-mollic soils, respectively (Table 2).

Table 2. Soil physicochemical parameters in relation to site and diagnostic category

Parameter	Mollic soil sites				Non-mollic soil sites			Sig. 2-tailed
	JM1	**JM2**	**JM3**	**SZP1**	**GBG**	**GUF**	**SZP2**	
BD ($g\ cm^{-3}$)	1.57b (0.04)	1.56b (0.02)	1.27a (0.03)	1.45b (0.11)	1.30e (0.03)	1.11d (0.04)	1.51f (0.04)	0.05*
MC (%)	16.51a (0.17)	18.31a (1.57)	28.58b (0.76)	16.57a (2.78)	15.05e (2.38)	16.36e (1.85)	9.43d (1.47)	0.00**
SOM (%)	2.44a (0.29)	3.39b (0.29)	3.90b (0.18)	1.84 (0.46)	3.66e (0.56)	3.71e (0.53)	0.98d (0.10)	0.84
pH-H_2O	7.80a (0.19)	7.41a (0.24)	7.78a (0.12)	7.51a (0.24)	4.07d (0.13)	5.49e (0.68)	8.47f (0.04)	0.04*
pH-KCl	7.14a (0.12)	6.76a (0.25)	7.14a (0.25)	6.90a (0.22)	3.17d (0.15)	4.55e (0.76)	7.89f (0.14)	0.04*
Ca^{2+} ($mg\ kg^{-1}$)	1588.0b (58.62)	901.66a (44.55)	1539.33b (82.44)	687.67a (296.02)	701.00e (208.01)	268.33d (164.83)	269.33d (65.16)	0.00***
Mg^{2+} ($mg\ kg^{-1}$)	38.53c (2.06)	30.63b (2.22)	39.67c (0.81)	24.90a (1.77)	32.37e (6.73)	20.37d (3.25)	12.17d (2.23)	0.01**
Na^{+} ($mg\ kg^{-1}$)	13.80c (1.57)	10.09ab (1.31)	6.78a (0.12)	11.07bc (1.67)	6.55e (1.02)	5.16d (1.25)	4.59d (0.17)	0.00***
K_2O ($mg\ kg^{-1}$)	235.33b (20.79)	500.44c (7.43)	533.44c (81.21)	117.88a (8.00)	256.22d (72.94)	99.17d (6.09)	80.66d (4.21)	0.00**
$CaCO_3$ (%)	0.00a (0.00)	0.00a (0.00)	1.52b (0.99)	0.29ab (0.51)	0.00d (0.00)	0.00d (0.00)	10.09e (7.06)	0.19
NO_3^--N ($mg\ kg^{-1}$)	7.01a (5.89)	9.51a (3.60)	4.87a (2.71)	5.70a (2.96)	29.73d (15.15)	14.33d (5.03)	9.22d (5.54)	0.03*
NH_4^+-N ($mg\ kg^{-1}$)	1.04a (0.13)	2.74ab (1.56)	5.09b (2.19)	4.76b (0.25)	6.53d (2.11)	6.59d (2.23)	3.31d (1.71)	0.05
P_20_5 ($mg\ kg^{-1}$)	140.33ab (27.49)	355.22bc (34.64)	504.56c (189.19)	49.83a (27.49)	87.90e (6.39)	17.28d (10.97)	159.89d (18.99)	0.01*
E_4/E_6	2.83a (1.21)	4.55b (0.19)	5.06b (0.32)	4.47ab (0.08)	5.95d (0.23)	6.10d (0.25)	6.43d (0.84)	0.00***

*Abbreviations: bulk density (BD), soil moisture content (MC), soil organic matter (SOM), Józsefmajor 1 (JM1), Józsefmajor 2 (JM2), Józsefmajor 3 (JM3), Gödöllő botanical garden (GBG), Gödöllő university forest (GUF), Szárítópuszta 1 (SZP1), Szárítópuszta 2 (SZP2). (n=9, mean (standard deviation)). Two separate ANOVA were performed, and means were compared. Different letters within row indicate significant differences at $p < 0.05$ with respect to site within diagnostic category. Sig. 2-tailed values show significant levels among the two diagnostic categories. *, **, ***: Significant at the 0.05, 0.01, and 0.001 levels, respectively*

The result of PCA showed that 36% and 25% of the total variance across sites were explained by PC1 and PC2, respectively. PC1 clearly separated mollic soils from non-mollic soils mainly based on Ca^{2+} (Figure 9). The high Ca^{2+} content in mollic soils could be associated to the base cation rich

parental materials mollic soils are formed on. The high BS (>50%) in surface soils is one of the diagnostic features of mollic horizon. In Chernozems, the BS percentage is close to 95 % with Ca^{2+} and Mg^{2+} as the main adsorbed cations (Driessen, 2001).

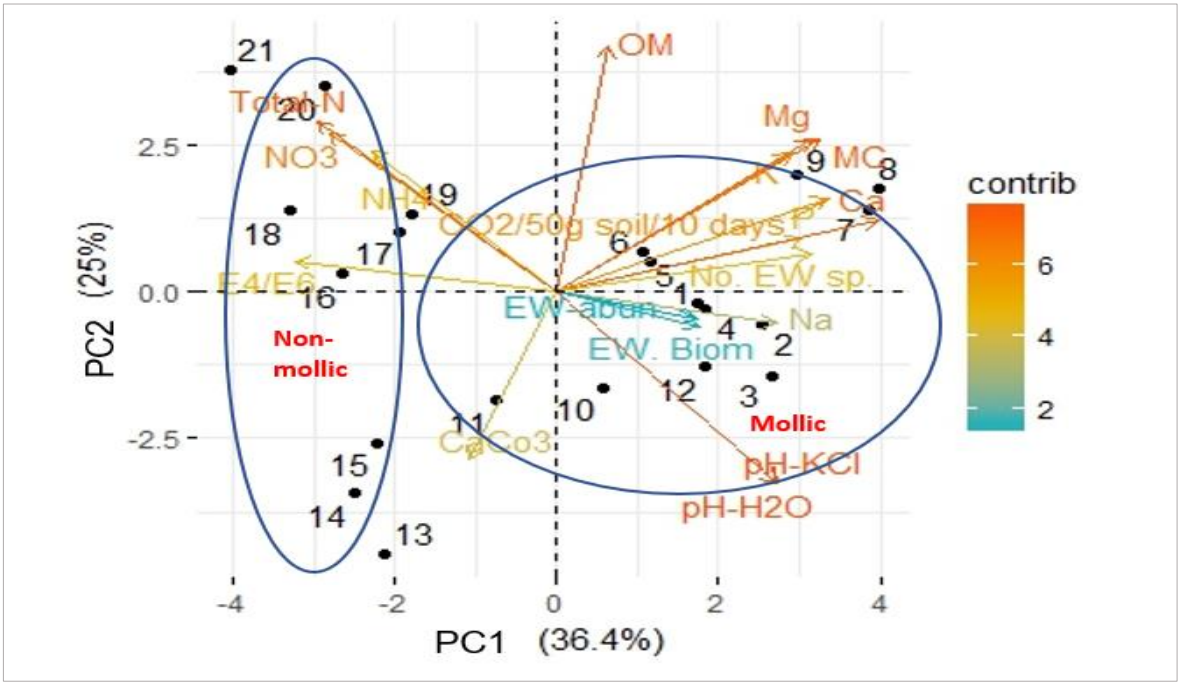


Figure 9. Principal component analysis showing the variance in soil properties across sites. Sites are coded in number (1-12 mollic soils and 13-21 non-mollic soils). Red color indicates high level of contribution whereas the blue color implies low contribution to the total variation.

4.1.2. Variation of SMR in relation to soil and LUT

SMR was significantly higher in mollic soils compared to non-mollic soils ($p < 0.01$). The mean SMR was recorded maximum in JM3 (26.77 mg CO_2 $50g^{-1}$ soil 10 $days^{-1}$) and minimum in SZP1 (6.78 mg CO_2 $50g^{-1}$ soil 10$days^{-1}$), higher in GBG and GUF (8.62 mg CO_2 $50g^{-1}$ soil 10 $days^{-1}$) and lower in SZP2 (4.40 mg CO_2 $50g^{-1}$ soil 10 $days^{-1}$) in mollic and non-mollic soils, respectively (Table 3). Different soil types have distinct physical and chemical properties that lead to different microbial communities and activities. It is well known that soil properties, such as pH, SOM, and C:N ratio are primary drivers of both SMR and microbial community composition (Moscatelli *et al*., 2018). Although both pH and SOM were positively correlated with SMR, available Ca^{2+} (r = 0.80), MC

(r = 0.72), and available Mg^{2+} (r = 0.69) were found to be strongly correlated with SMR (Table 4). Based on the result of PCA, the concentration of Ca^{2+} was the key contributing factor in explaining the total variation in the sites. Accordingly, the SMR significantly separated mollic soils from non-mollic soils, primarily on the bases of higher Ca^{2+} (Figure 9). Ca^{2+} is a vital soil macronutrient

which may enhance the mineralization of SOM by affecting its labile fractions (Kužel *et al.*, 2010). In Hungary, Filep and Szili-Kovács (2010), with a controlled pot experiment, found that soil respiration was higher in soils limed with $CaCO_3$.

Table 3. Soil biological properties in relation to site and diagnostic category

Parameter	Mollic soil sites				Non-mollic soil sites			Sig. 2-tailed
	JM1	JM2	JM3	SZP1	GBG	GUF	SZP2	
SMR ($mgCO_2$ 50 g^{-1} $soil10days^{-1}$)	19.80ab (9.19)	11.73a (3.90)	26.77b (3.22)	6.78a (1.68)	8.62d (3.32)	8.62d (2.08)	4.40d (1.65)	0.01**
EW abundance (ind. m^{-2})	16.00a (27.71)	90.67ab (75.61)	133.33ab (40.26)	336.00b (216.44)	10.67d (18.47)	42.67d (40.26)	0.00d (0.00)	0.02*
EW biomass (g m^{-2})	7.87a (13.63)	10.22a (9.75)	44.67a (15.09)	111.39a (83.67)	1.00d (1.71)	6.84d (7.38)	0.00d (0.00)	0.03*
Species richness of EW	0.33a (0.33)	0.67a (0.33)a	2.00a (0.57)	1.00a (0.57)	0.00d (0.00)	0.33d (0.33)	0.00d (0.00)	0.01**

Abbreviations: soil microbial respiration (SMR), earthworm (EW), Ind. (Individual). Józsefmajor 1 (JM1), Józsefmajor 2 (JM2), Józsefmajor 3 (JM3), Gödöllő botanical garden (GBG), Gödöllő university forest (GUF), Szárítópuszta 1 (SZP1), Szárítópuszta 2 (SZP2). (n=3, mean (standard deviation)). Two separate ANOVA were performed, and means were compared. Different letters within row indicate significant differences at p < 0.05 with respect to site within diagnostic category. Sig. 2-tailed values show significant levels among the two diagnostic categories (, **, ***:at 0.05, 0.01, and 0.001, respectively).*

In line with Bååth and Anderson (2003), the present study found a positive correlation between pH and SMR (r = 0.23), although it was not significant. However, Creamer *et al*. (2016a), studying the potential microbial activity of European soils across a wide range of physicochemical parameters, contrasting biogeographical (climatic) zones and land uses, showed a significant negative correlation of pH with basal respiration. The contradicting result may be due to the difference in the spatial scale considered.

There was a negative correlation between available nitrogen (NO_3^--N and NH_4^+-N) and microbial respiration. Similar observation was made by Gangwar *et al.* (2018) where NO_3^--N was significantly negatively correlated with SMR in salt affected soils (Solonetz) of Hungary. Kaštovská *et al*. (2010) suggested N application may inhibit the biological activity of soil microbes and reduces SMR rates.

The mean basal respiration did not show significant difference between LUTs within mollic diagnostic category ($p < 0.05$), but it differed within non-mollic category ($p < 0.05$) (Figure 10).

Table 4. Correlation matrix between physicochemical and biological properties

Parameter	SMR	Earthworm biomass	Earthworm abundance
EW. Biomass	-0.013		
EW. Abundance	-0.045	0.965***	
BD	-0.073	-0.016	-0.010
Ca^{2+}	0.801***	0.184	0.138
$CaCO_3$	-0.258	-0.161	-0.21
E_4/E_6	-0.435*	-0.215	-0.217
MC	0.718**	0.326	0.338
SOM	0.418	-0.12	-0.052
pH-H_2O	0.231	0.250	0.237
pH-KCl	0.235	0.250	0.239
Mg^{2+}	0.699**	0.087	0.073
Na^+	0.232	0.317	0.321
K_2O	0.647**	-0.056	0.000
NO_3^--N	-0.293	-0.268	-0.277
NH_4^+-N	-0.191	0.064	0.095
P_2O_5	0.6277*	0.016	0.000

*Pearson's correlation p<0.05 (n=3). *, **, ***: Significant at 0.05, 0.01, and 0.001 levels, respectively.*
Abbreviations: bulk density (BD), soil organic matter (SOM), soil moisture content (MC)

The mean SMR was highest in grassland followed by arable soils of Józsefmajor, whereas the lowest value was recorded in arable soils of Szárítópuszta. This study found a significant variation of SMR among soils of different textures ($p < 0.001$), with the highest value in silty clay loam (SiCL) soils and lowest in sandy (S) soils (Figure 11). Soil texture is an important physical property that strongly influences water and nutrient availability in the soil by affecting pore size distribution and surface area. Fine textured soils have a large surface area which allows the soil to hold more nutrients and water that could enhance the microbial activity and in turn increases carbon mineralization (Hamarashid *et al*., 2010).

The SMR was more pronounced in mollic soils than non-mollic soils irrespective of the LUTs, suggesting soil type rather than LUT might be the dominant driver of basal microbial respiration in the study area. These findings correspond with the work of Katulanda *et al*. (2018), who stated that although land use had impact, the inherent soil properties had a greater effect on soil microbial abundance and therefore on SMR.

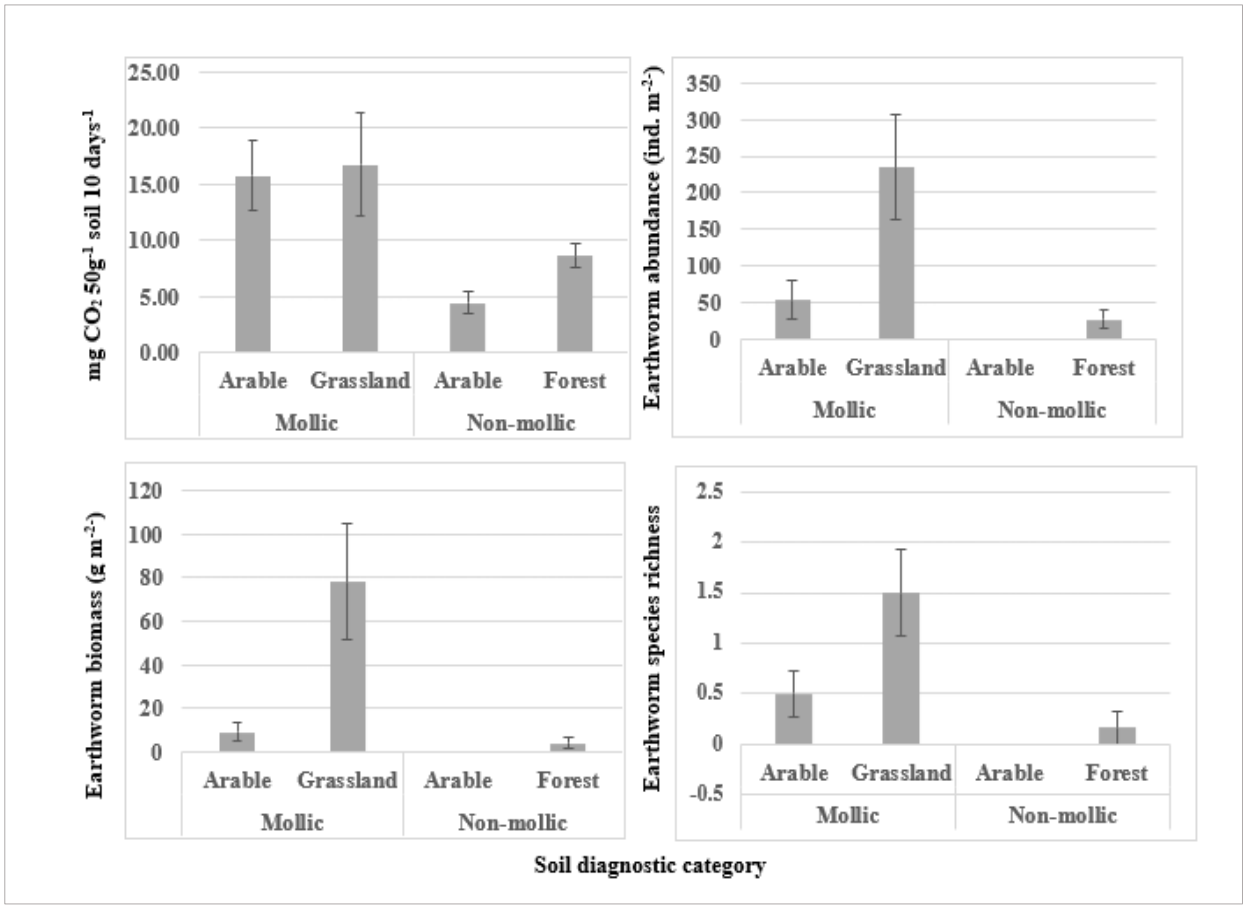


Figure 10. Effect of soil and LUT on SMR and earthworm (abundance, biomass, and species richness) (mean of three measurements and upper and lower error bars showing confidence interval).

The correlation test showed that there was a positive correlation of SMR with P_2O_5 and K_2O. In southern China, Liu *et al*. (2013) noted that after long-term available P addition in N-saturated old-growth tropical forest, SMR was significantly increased, implying the addition P increases labile C by releasing organic matter bound to the sorption sites. Studies documented that SOM greatly influence the soil microbial activity (Moscatelli *et al*., 2018), however, this study did not find a strong correlation between these parameters. The reason could be that the positive effect of SOM on SMR might be masked by the negative effect of a low pH on SMR (Creamer *et al*., 2016a) as the highest SOM was recorded in forest soils where the pH was lowest. The low pH in forest soils could negatively affect the microbial activities and consequently decrease the rate of microbial decomposition (Moghimian *et al*., 2017). Further, the litter quality of forests i.e., high C:N and lignin:N ratio, which is less decomposable, may also play a role for the low microbial respiration in the forest soils (Solly *et al*., 2014).

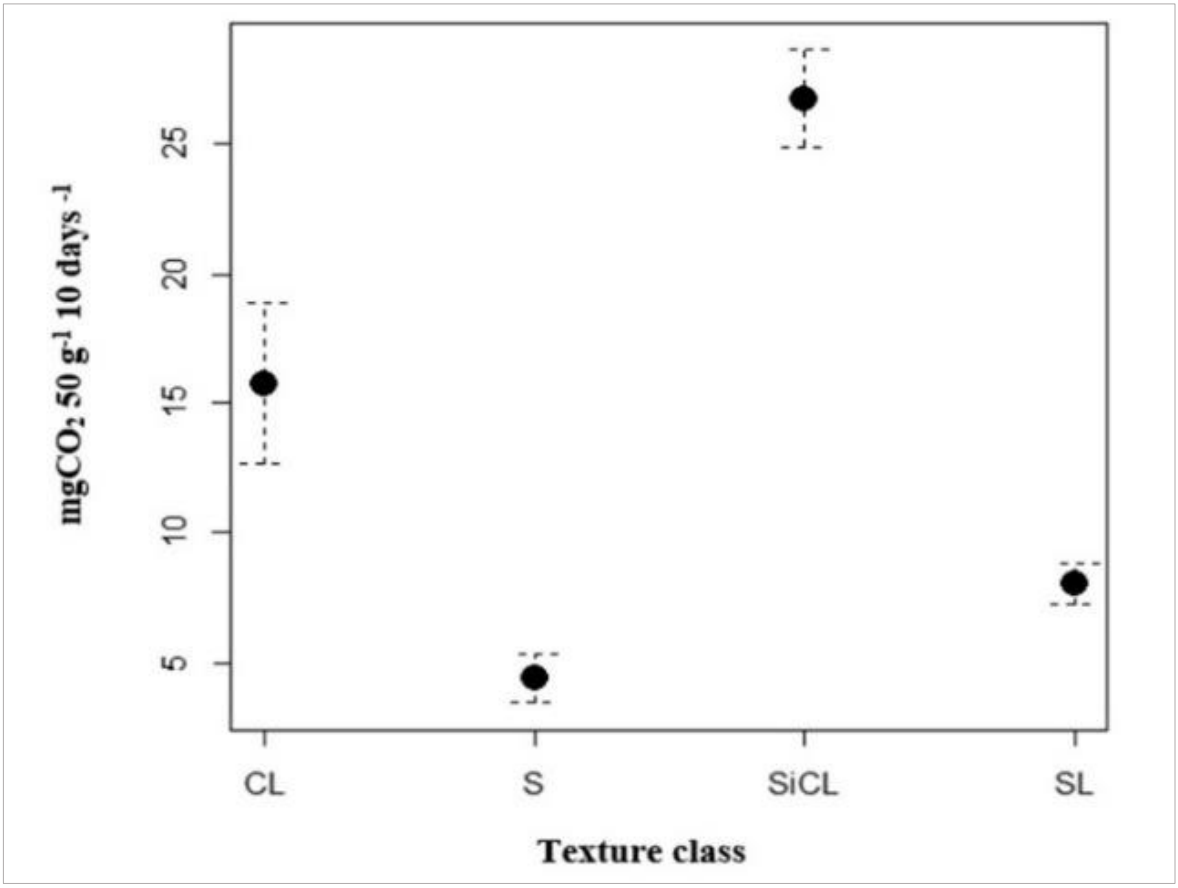


Figure 11. Mean and standard error of SMR across soil texture classes: CL (Clay Loam), SiCL (Silty Clay Loam), SL (Sandy Loam), and S (Sand).

4.1.3. Patterns of earthworm communities across soil and LUTs

Earthworm abundance and biomass were significantly higher in mollic soils, compared to non-mollic soils ($p< 0.01$). Earthworm abundance ranged from 16.00 to 336.00 ind m^{-2} and from 0 to 42.67 ind m^{-2}, earthworm biomass from 7.87 to 111.39 g m^{-2} and from 0 to 6.84 g m^{-2} in mollic and non-mollic soils, respectively. In total five earthworm species were identified in mollic soils whereas only one species of earthworm was found in non-mollic soils. The supply of organic matter in the soil is a key driver of earthworm abundance, as earthworms feed on either poorly decomposed litter at the soil surface or ingest soil and assimilate a small fraction of organic matter it contains (Bertrand *et al.*, 2015). However, this study did not find a strong positive correlation of SOM with earthworm abundance and biomass. Similarly, on the arable soils in France, Pelosi *et al*. (2009) studied earthworm abundance, biomass, and diversity between conventional and organic farming for three years found no significant variation between the two management systems despite high SOM in organic farming system. Generally, earthworms prefer to feed small particle sized over large particle sized organic matter (Lowe and Butt, 2003); organic matter with low C:N ratio over high C:N ratio (Solly *et al*., 2014). Most of the non-mollic soils in the study area are under forest cover where the C:N ratio of the organic matter expected to be high (less humified).

Litter with high C:N ratio is less palatable for the earthworms, affecting the earthworms feeding activity, might cause the low earthworm population in non-mollic soils (Ernst *et al*., 2009). Absence of a significant effect of organic matter on earthworm abundance and biomass in the study area could also be the presence of earthworms in the study area depended on factors more important than organic C, and when present, organic C is consumed by earthworms. There was a negative correlation between NO_3^--N with both earthworm abundance and biomass. Studies have shown that earthworms can increase the leaching of mineral N and P because of their effects on soil structure (Blouin *et al*., 2013). In this study, earthworm abundance and biomass were generally high in site with high pH. Generally, this study did not find strong association of earthworm abundance and biomass with any of the investigated physicochemical properties (Table 4).

Earthworm abundance, biomass, and species richness were greater in grassland than arable land in mollic soils and forest compared to arable land in non-mollic soils (Figure 10). The absence of soil tillage coupled with relatively high availability of organic matter in grassland sites may be the reason for the occurrence of high earthworm communities in the grassland sites. A similar observation was made by Cluzeau *et al*. (2012) that the earthworm density, biomass, and species richness were highest in grasslands compared to forest and arable lands. In all sites, the earthworm communities were predominantly dominated by juveniles. A total of five species were identified, i.e., *Aporrectodea caliginosa* (Savigny, 1826), *Octolasion lacteum* (Örley, 1881), *Aporrectodea rosea* (Savigny, 1826), *Proctodrilus opisthoductus* (Zicsi, 1985), and *Aporrectodea georgii* (Michaelsen, 1890). The grassy Chernozem (JM3) soils had the highest number of earthworm species (4 out of 5). *Aporrectodea caliginosa* was the most abundant earthworm species in the study area. It belongs to the endogeic group and well adapted to pastures, gardens, forest, and even in the poorest sandy soils (Csuzdi and Zicsi, 2003). *Proctodrilus opisthoductus, Aporrectodea georgii*, and *Octolasion lacteum* were only found in the areas of grassland. The earthworm community majorly constituted the juveniles' category across all soil and land-use type. Similar result was reported by Kamdem *et al*. (2018). The ratio of juveniles was higher in non-mollic (90%) compared to mollic soils (81%), and in forest (90%) compared to arable (88%) and grassland (64%) soils, respectively. Studies documented that the quality of food material affects not only the size of population but also the species present and their rate of growth. Earthworms gain less biomass and mature more slowly when fed with oak leaves (Penning and Wrigley, 2018), this could be the reason why the ratio of juveniles was higher in forest soils compared to other LUTs.

In this study, lacking explicit association of earthworms (abundance, biomass) and other soil properties implies that agricultural practices related to tillage might have a profound effect on

earthworm communities than soil properties. Continuous tillage in arable lands may result in high BD, low SOM, and low MC which collectively influence the earthworm communities. Crittenden *et al*. (2014) noted that tillage and farming system explained a significant proportion of total variation in earthworm abundance after studying the effects of tillage systems on earthworm populations in conventional and organic farming in both short-term (15 days) and medium term (3 years) study in The Netherlands. In their review article, Birkás *et al*. (2010) reported that earthworm live weight in soil under direct till was five times greater than in soil under ridge till and three and half times greater than in soil under conventional tillage in Hungary, implying the importance of tillage system in determining the earthworm biomass. Soil texture is one of important soil properties that influence earthworm communities. Sandy soils are unsuitable for earthworm inhabitation either because the abrasive action of sand grains damages earthworms' cuticle, or because these soils dry out more easily and poor in nutrient and SOM (Sankar and Patnaik, 2018). In agreement with that, this study did not find any earthworms in SZP2 site that had sandy texture.

Earthworms accelerate SOM decomposition by stimulating SMR and by fragmentizing, ingesting, and transporting fresh plant material into the soil (Bertrand *et al.*, 2015). Earthworms may also affect the SMR by controlling the biomass and/or activity of microbiota and, further, to mineralize/stabilize microbial products (Huang *et al*. 2015). However, this study did not find any significant correlation between earthworm (abundance, biomass, and species richness) with SMR (Table 4). The mechanisms through which earthworms affect SMR is species-specific and the overall effects could be positive, negative, and neutral (Ernst *et al*., 2009). Studies reported that earthworms induced short-term increase of soil respiration, followed by gradual decease back towards the baseline (Chang *et al*., 2016). The finding of this research was collaborating with the findings of Chang *et al*. (2016) and Fisk *et al*. (2004) that showed no effect of earthworm on soil respiration. Overall, this study highlighted that LUT related to tillage could be a more powerful variable than soil properties in explaining earthworm communities in the study area.

4.2. Influence of depth on soil chemical properties and SMR

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

4.2.1. Soil depth and soil chemical properties

The analytical results of chemical properties are presented in Table 5. In all sites, the SOM was higher in surface than subsurface soils, although a significant difference between the two depths was only observed in KAMG site. SOM ranged between 3.63 to 39.53 % in surface and from 3.6 to 10.38% in subsurface layers, respectively. Enhanced SOM accumulation in the topsoil is attributed to the continuous input of organic matter from plant and animal residues as well as root exudates that increases the mineralization and accumulation of organic matter (Kunlanit *et al*., 2019). Similarly, Liu *et al.* (2018a) and Tufa *et al*. (2019), reported that the SOM greatly decreased with increasing soil depth, implying the strong influence of soil depth on SOM. The study did not find a significant decrement of soil pH with soil depth, however, in both sampled depths, the soil pH was significantly lowest in KAMG and highest in SZHE. In line with this, Liu *et al.* (2018a), found a minor difference of soil pH at 0–10 cm, 10–30 cm, and 30–60 cm depth. On the other hand, soil depth had a strong effect on total N (mg kg^{-1}), NO_3^--N (mg kg^{-1}), and NH_4^+-N (mg kg-1) ($p = 0.00004$, 0.0005, and 0.02, respectively), and significantly higher in surface soils compared to subsurface soils. This finding also corroborates with previous studies that documented the substantial decrease of available N with soil depth gradients (Ouyang *et al.*, 2017; Liu *et al.*, 2018a). It is a well-established fact that the availability of nutrients, such as available N and P are closely linked to the availability of SOM (Jobbágy and Jackson, 2001).

A reverse trend indicating an increase of AL-P_2O_5 with soil depth in all sites except KAMG was noted in comparison to a decrease of E_4/E_6. The value of E_4/E_6 was significantly lower, in both depths, in CSOB grassland site compared to the two forest sites. The finding of increasing AL-P_2O_5 with depth in this study contradict the findings of Jobbágy and Jackson (2001), who investigated the vertical distribution of global soil nutrients in the top meter of soil, fixed depth intervals of 20 cm, using National Soil Characterization Database (NSCD) of the United States Department of Agriculture found a significant decrement of AL-P_2O_5 along soil depth gradient. The probable reason for the contradicting results might be associated with the difference in soil depth considered. In general, all exchangeable base cations (Ca^{2+}, Mg^{2+}, K^+, and Na^+) showed similar declining patterns in the subsoils from the surface soils. This could be either local enrichment of base cations from the parent rock or addition through plant and animal residues in surface soils (Korkanc *et al*., 2014). Similar patterns of decrement of base cations along the soil depth were observed by Tufa *et al*. (2019). CEC showed a significant difference among soil depth ($p = 0.005$) and ranging between 44.25 to 77.63 cmol kg^{-1} in surface soils and between 32.13 to 62.00 cmol kg^{-1} in subsoils. The surface soils had significantly

Table 5. Soil chemical properties at two depths along the three sites

Parameter	Site					
	SZHE		CSOB		KAMG	
	0-10 (cm)	10-25 (cm)	0-10 (cm)	10-25 (cm)	0-10 (cm)	10-25 (cm)
SOM (%)	3.63a (0.03)	3.66a (0.11)	11.59b (0.63)	10.38b (0.17)	39.53c (0.98)	5.87a (0.01)
pH (H_2O)	7.80d (0.00)	8.00e (0.00)	6.40b (0.00)	6.50c (0.00)	3.80a (0.000)	3.80a (0.00)
$CaCO_3$ (%)	5.59b (0.00)	13.46c (0.00)	0.00a (0.00)	0.00a (0.00)	0.00a (0.00)	0.00a (0.00)
NH_4^+-N (mg kg^{-1})	7.25ab (0.72)	5.45a (0.14)	11.70b (0.06)	8.30ab (1.33)	68.15c (2.18)	9.92ab (0.28)
NO_3^--N (mg kg^{-1})	5.50cd (0.81)	3.70bc (0.29)	7.20d (0.29)	0.00a (0.00)	21.14e (1.45)	0.77ab (0.00)
Total N (mg kg^{-1})	12.75b (0.09)	9.15a (0.14)	18.90c (0.35)	8.30a (1.33)	89.28d (0.73)	10.69ab (0.28)
AL-P_2O_5 (mg kg^{-1})	24.05a (1.53)	26.70a (0.92)	772.50c (10.68)	1229.50d (41,86)	212.00b (6.35)	108.50a (4.33)
AL-K_2O (mg kg^{-1})	73.70ab (6.70)	60.10a (1.33)	503. 00e (9.24)	395.50d (1.44)	245.00c (21.36)	112.35b (7.03)
E_4/E_6	7.86c (0.00)	6.99b (0.41)	4.74a (0.11)	4.38a (0.00)	6.29b (0.00)	6.32b (0.00)
Ca^{2+} (cmol kg^{-1})	23.59d (0.17)	20.91c (1.20)	27.94e (0.22)	27.25e (0.11)	9.93b (0.13)	0.19a (0.01)
Mg^{2+} (cmol kg^{-1})	0.91c (0.00)	0.59b (0.05)	3.39e (0.02)	2.67d (0.02)	3.91f (0.00)	0.45a (0.00)
K^+ (cmol kg^{-1})	0.03a (0.00)	0.01a (0.00)	1.32e (0.02)	0.85c (0.00)	0.94d (0.00)	0.15b (0.00)
Na^+ (cmol kg^{-1})	0.12ab (0.00)	0.08ab (0.05)	0.18bc (0.01)	0.13ab (0.00)	0.26c (0.01)	0.06a (0.01)
CEC (cmol kg^{-1})	44.25b (0.34)	38.46b (0.36)	65.05c (0.69)	62.00c (1.49)	77.63d (0.22)	32.13a (2.48)
BS (%)	55.58c (0.79)	56. 21c (3.88)	50.50c (0.96)	49.95c (1.42)	19.36b (0.12)	2.66a (0.12)

Values are the means (Standard errors) of three replicates, different letters across the rows indicate significant differences among soil depths and sites at $p < 0.05$ (ANOVA).

Abbreviations: soil organic matter (SOM), cation exchange capacity (CEC), base saturation (BS), Szárhalmi forest (SZHE), Csobánc (CSOB), Károly-magaslat (KAMG)

higher CEC than the subsoil probably because of high SOM content in surface soils. A significant lowest value of BS was observed in KAMG site and it decreased with soil depth in all sites, however, the decrement was not significant ($p = 0.6$). $CaCO_3$ was only present in SZHE site and it was higher in surface than subsurface layer.

Overall, among the investigated chemical attributes, available nitrogen, CEC, Na^+, and Mg^{2+} showed a strong decrement along the depths. As Jobbágy and Jackson (2001) explained, nutrients that are most limiting for plants (N, P, Ca, Mg) are present in high amount in the shallowest depth of the soil as these elements moved upward by biological cycling when plants absorb and transport them aboveground and recycle to the soil surface by litterfall and throughfall.

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

The rate of soil respiration ranged from 25.44 in SZHE to 84.59 mg CO_2 $50g^{-1}$ soil $10days^{-1}$ in KAMG and from 16.31 in KAMG to 17.78 mg CO_2 $50g^{-1}$ soil $10days^{-1}$ in SZHE in surface and subsurface layers, respectively.

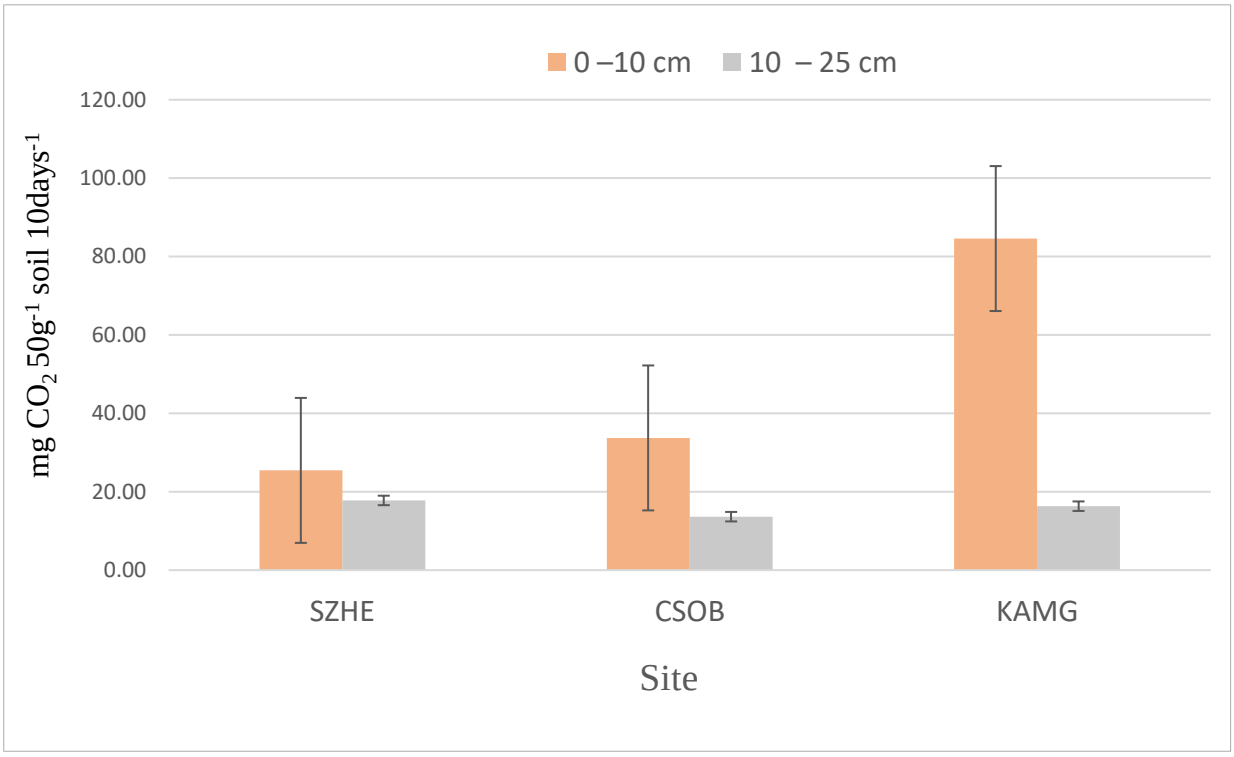


Figure 12. SMR measured at two soil depths across three sites (mean of three measurements and upper and lower error bars showing confidence interval).
Szárhalmi forest (SZHE), Károly-magaslat (KAMG), Csobánc (CSOB).

This study found that SMR was significantly different among the two soil depths ($p=0.0005$) and decreased with increasing soil depth (Figure 12). This agrees with Fang and Moncrieff (2005),

who assessed the variation of SMR with depth in relation to soil carbon composition in Scotland, finding the highest respiration in the 0–8 cm layer that accounted more than 50% of total respired CO_2 from the whole profile. Similarly, Dos Santos Soares *et al.* (2019) investigated the effects of various cropping systems on chemical and microbiological soil properties in long-term no-tillage systems along 0–10, 10–20, and 20–30 cm depth found that basal respiration was significantly higher in 0–10 cm of the soil in two out of three investigated cropping systems. Liu *et al.* (2018a) studying the impact of LUT and soil depth on physicochemical and biological properties in China, agree with this finding, stating that basal respiration was the most discriminating variables for soil quality across soil depths than LUTs.

Table 6. Pearson's correlation coefficient between chemical soil properties and SMR

Soil chemical properties	SMR
SOM (%)	0.946***
pH(H_2O)	-0.518*
$CaCO_3$ (%)	-0.214
NH_4^+-N (mg kg^{-1})	0.963***
NO_3^--N (mg kg^{-1})	0.974***
Total N (mg kg^{-1})	0.980***
AL-K_2O (mg kg^{-1})	0.142
AL-P_2O_5 (mg kg^{-1})	-0.183
E_4/E_6	0.062
Ca^{2+} (cmol kg^{-1})	-0.251
Mg^{2+} (cmol kg^{-1})	0.692**
K^+ (cmol kg^{-1})	0.448
Na^+ (cmol kg^{-1})	0.823***
CEC (cmol kg^{-1})	0.730***
BS (%)	-0.325

, **, * represent significance level at 5% ($p < 0.05$) and 1% ($p < 0.01$), 0.1% ($p<0.001$) respectively. Abbreviations; soil organic matter (SOM), cation exchange capacity (CEC), and base saturation (BS)*

SMR is regulated by soil chemical properties, among which, organic matter, pH, and available nutrients (Batubara *et al.*, 2019). These soil properties substantially vary along soil depths (Zhang *et al.*, 2017b) and the change in chemical attributes lead to a shift in microbial communities, thereby microbial respiration (Liu *et al.*, 2018b). In this study, the variation of SMR was in

accordance with the change in available N and SOM along the two soil depths. Accordingly, SMR had a significant positive correlation with total N, NO_3^--N, NH_4^+-N, and SOM, respectively (Table 6). Further, the PCA shows that PC1explained 51.1% of the total variation, and SOM, CEC, SMR, and available N were the most prominent predictors, respectively. The second PC accounts for 28.6% of the variation, and P_2O_5 was the key contributor (Figure 13). Previous studies documented that SOM, available N (Liu *et al.*, 2018a), and CEC (Adugna and Abegaz, 2015) primarily influence the metabolic activity of soil microbes, confirming the finding of this study.

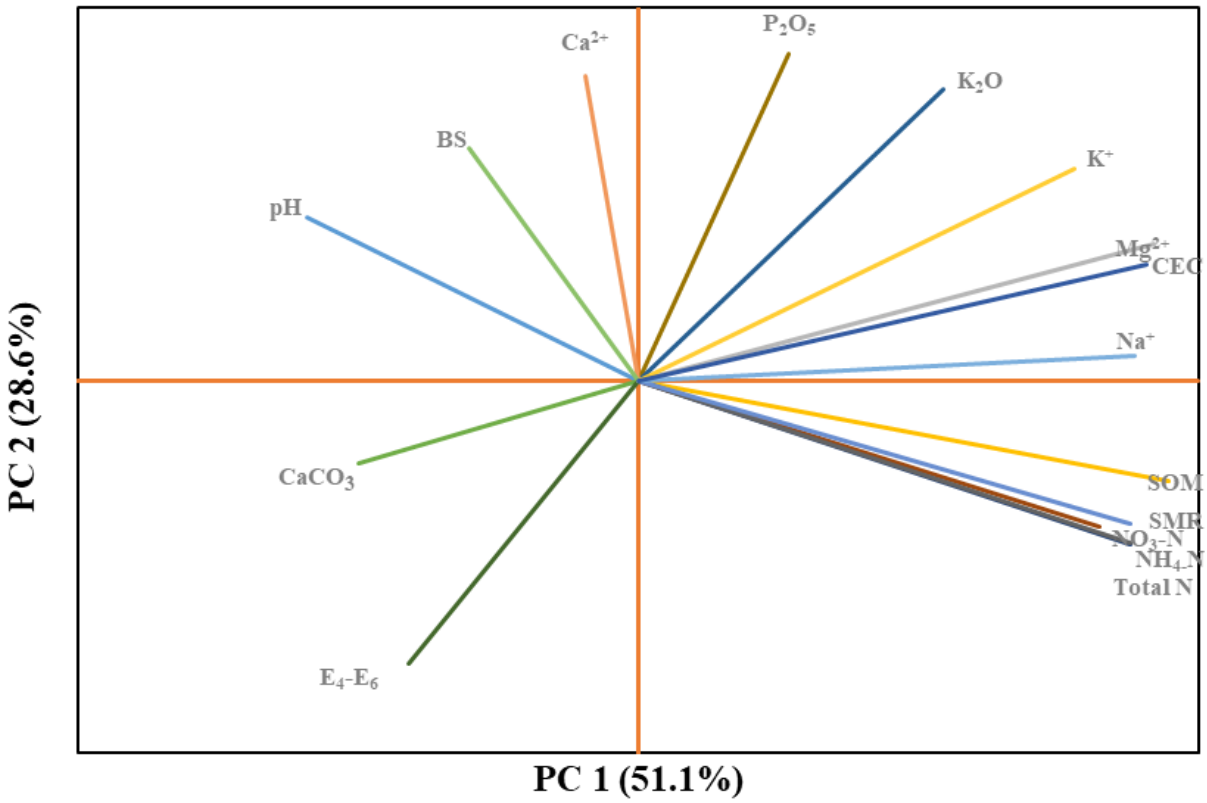


Figure 13. Correlation monoplot showing the relationship between soil properties. Small angle between the soil properties represents positively correlated, an angle of 90^o and close to 180^o indicate the variables are not correlated and negatively correlated respectively, and the length of the line and its closeness to the square, represent how well the variable is represented in the plot (short and far line implies poor representation).

SOM significantly influences microbial respiration as it provides energy and nutrients for microbes. Most of the organic matter in the form of litter, animal residues, and root exudate is higher in the surface than the subsurface (Jobbágy and Jackson, 2001) and this could enhance the rate of microbial respiration in the surface. Total organic matter in the soil is substantially related

to quality and availability of nutrients, such as nitrogen. Furthermore, it also linked with CEC as organic matter in soil provides colloids with a high capacity for cation exchange (Nguyen and Marschner, 2017).

The correlation of organic matter with N in relation to microbial respiration is well recognized (Richter *et al.*, 2018). In this study, a strong positive correlation was evident between these chemical variables and with SMR (Figure 13). Similar observations of a positive effect of nitrogen (Deng *et al.*, 2010), organic carbon (Creamer *et al.*, 2016) and CEC (Adugna and Abegaz, 2015) on SMR were made, implying these parameters could be major drivers of soil microbial activity.

In a range of previous studies, pH has shown to be a significant soil property driving microbial respiration (e.g., Wakelin *et al.*, 2008; Andruschkewitsch *et al.*, 2014). Soil pH influences the solubility of SOM and changes the rate of microbial carbon turnover. It also affects the availability and distribution of nutrients which are important for microbes to decomposed SOM (Ebrahimi *et al.*, 2019). This study found a significant negative correlation of pH with SMR ($p < 0.05$, $r = -0.51$). This result contradicts the finding of Ebrahimi *et al.* (2019), who documented the neutral correlation between soil pH and basal respiration but in agreement with Creamer *et al.* (2016a) who noted a significant negative correlation of basal respiration with pH, after assessing the microbial respiration profile across Europe using MicroResp^{TM} method. Generally, the results evidently show that the rate of microbial respiration was greatly influenced by soil depth and depending highly on the amount of SOM and available N as revealed by their similar patterns along the soil depth and their strong positive relation.

4.3. Response of soil physicochemical properties and SMR to different LUTs

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

4.3.1. Soil physicochemical properties among LUTs

Considerable variability was observed among the LUTs with respect to most physicochemical properties (Table 7). Soils from the arable land showed significantly lower concentration of SOM with mean of 2.83% compared to soils collected from forest (4.85%) and grassland (5.06%), respectively. As highlighted by Rodrigues *et al.* (2017), tillage practices in arable land may result in losses of carbon from the soil due to decomposition, erosion, and leaching. Similarly, Celik (2005) found that compared to forest and pasture soils, SOM in cultivated soils decreased by 44 and 48%, respectively for the top 0-10 cm layer over 12 years in southern Mediterranean highland of Turkey. Likewise, Kunlanit *et al*. (2019), investigated the influence of land use change on SOC

stock and their quality in Northeast Thailand, found that the conversion of forest to cultivated land significantly reduced both the stock of SOC and humic acid. The ratio of E_4/E_6 was higher (6.31) in forest compared to arable (4.58) and grassland (3.75), implying forest soils contained fulvic acid whereas grassland and arable soils had humic acid (de Melo *et al*., 2016).

Table 7. Summary of statistics of soil physicochemical properties across three land use types

Parameter	LUT			p-value
	Forest	Grassland	Arable	
SOM (%)	4.85b (0.280)	5.06b (0.58)	2.83a (1.19)	0.031 *
pH(H_2O)	5.08a (0.38)	7.62b (0.08)	7.83b (0.13)	0.002**
pH(KCl)	4.03a (0.33)	6.58b (0.08)	6.67b (0.16)	0.003**
$CaCO_3$ (%)	0.00a (0.00)	0.23ab (0.11)	1.14b (0.51)	0.112
MC (%)	21.58a (2.89)	31.20a (4.85)	21.98a (4.44)	0.241
NH_4^+-N (mg kg^{-1})	9.00b (0.47)	7.81ab (0.57)	5.96a (0.58)	0.004 **
NO_3^--N (mg kg^{-1})	30.08b (3.66)	8.779a (2.62)	0.44a (0.20)	0.000***
Total N (mg kg^{-1})	39.08c (3.22)	16.57b (2.22)	6.40a (0.39)	0.000***
AL-K_2O (mg kg^{-1})	143.00a (12.92)	199.00b (8.19)	135.75a (8.07)	0.000 ***
AL-P_2O_5 (mg kg^{-1})	34.15a (3.99)	58.27a (21.14)	104.17a (32.82)	0.281
Ca^{2+} (cmol kg^{-1})	5.75a (1.24)	13.56b (2.15)	14.99b (2.06)	0.002**
Mg^{2+} (cmol kg^{-1})	0.92a (0.24)	7.38b (2.87)	2.46ab (0.02)	0.150
K^+ (cmol kg^{-1})	0.25a (0.04)	0.35a (0.02)	0.45b (0.05)	0.003 **
Na^+ (cmol kg^{-1})	0.08a (0.02)	0.64a (0.28)	0.20a (0.03)	0.430
CEC (cmol kg^{-1})	33.77ab (0.60)	35.88b (4.42)	30.71a (5.31)	0.750
BS (%)	20.61a (4.09)	56.55b (7.94)	60.12b (3.88)	0.003
E_4/E_6	6.31b (0.10)	3.57ab (0.68)	4.58a (0.39)	0.044

*Values are the means (standard errors) of six replicates; different letters across the rows indicate significant differences among LUTs at p < 0.05 (ANOVA). p-values show significant levels among the LUTs. *, **, ***: Significant at the 0.05, 0.01, and 0.001 levels, respectively. Abbreviations: land use types (LUTs), soil organic matter (SOM), soil moisture content (MC), cation exchange capacity (CEC), base saturation (BS)*

The differences between LUT in K_2O was significant ($p < 0.001$), ranging from 135 in arable land to 199 mg kg^{-1} in grassland. Grassland soils had higher K_2O than forest and arable land, possibly due to little soil disturbance and high OM which may resulted in high nutrient adsorption and low leaching rate. Rodrigues *et al.* (2017) recorded that potassium concentration in minimum tillage was significantly higher than that of conventional tillage system. Significant differences in available form of N (NH_4^+-N, NO_3^--N, and total N) were detected between LUT. The available nitrogen content of soils under cultivation was lower compared to levels in the forest and grasslands. This finding collaborated with Gol (2009), who reported that conversion of forest to cultivated land significantly decreased both the concentration and stock of SOM and TN in

Dagdami river catchment located in the highlands of the Black Sea region of Turkey. Higher litter production and N fixation by the different tree and shrub species within the forest probably contributed to higher available N content in forested soil. Equally, loss of available N through faster decomposition of organic matter associated with continuous tilling of soils could also be a reason.

Forest soils exhibited significantly lower soil pH as compared to grassland and arable soils. However, the difference in pH between the grassland and arable soils was not statistically significant. The low pH in forest could be attributed to low base cations in the soil or the type of litter residues of the forest vegetation. This finding is consistence with the finding of Rodrigues *et al*. (2017), who noted higher soil pH value in pasture soils compared to forest soils. In comparison with grassland and forest soils, there was a high concentration of $CaCO_3$ and P_2O_5 in arable soils, and this might be due to the application of inorganic fertilizers and liming. The high content of P in arable soils was confirmed by Maharjan *et al*. (2018), who noted a significant increment of P stock by 64% and 36% at 0–10 cm and 10–20 cm depth, respectively in conventional farming compared to forest. The significant low content of P in forest soils could be associated with the low pH in the forest. Various studies documented that under low pH, P could be fixed with Al or Fe, thus became unavailable. MC did not show significant different among the LUTs, whereby the highest value recorded in grassland soils as compared to arable land and forest soils. The relatively high content of SOM could be one of the reasons for the high soil MC in grasslands. It has been shown that SOM increases water holding capacity and infiltration of the soil by improving soil porosity and reduce soil compactability (McCauley *et al*., 2005).

Among the base cations, Ca^{2+} and K^{+} were significantly higher in arable soils followed by grassland and forest soils respectively, whereas the amount of Mg^{2+} and Na^{+} were highest in grassland and lowest in forest. The high content of Ca^{2+} in cultivated soils may be attributed to the high $CaCO_3$ content in the soils. In general, all the base cations were lower in forest soil, owing to acidic condition of forest soils, facilitating leaching of these cations. The CEC did not show a significant difference between the LUTs and ranged from 30.7 to 35.8 cmol kg^{-1} in arable and grassland soils, respectively. The forest soils significantly differed in BS from both the grassland and arable soils; however, there was no significant difference in BS between arable and grassland soils. The higher BS in arable land probably due to high content of Ca in the soils as exchangeable complex of the investigated soils was dominated by Ca^{2+}.

4.3.2. Influence of LUT on SMR and major drivers for its variability

Based on Kruskal-Wallis test, SMR significantly differed among the LUTs (p= 0.003). The grassland showed significantly higher SMR compared to the forest and arable land (Figure 14). This finding agrees with Creamer *et al.* (2016a) who reported that grassland soils had a remarkable high rate of SMR than arable soils; however, it was in contrast with Liu *et al.* (2018b), who found that farmland had higher basal respiration compared to orchard, grassland, and abandoned land. As highlighted by Liebig (1996), SMR is an important indicator for soil health since it indicates the level of microbial activity, SOM content, and its decomposition. Usually, higher SMR reflects high below ground microbial activity (Ryan and Law, 2005). One of the possible reasons for the high SMR in grassland soils could be a high level of labile C in the grassland system. SOM/SOC has been well documented to affect soil respiration as it is the primary energy source for microbes (Creamer *et al*., 2014). In line with that, this study also found a significant positive correlation of SMR with SOM (r = 0.56; p = 0.01483) (Table 8). In their study, Liu *et al.* (2018b), discussed that soil respiration rate was related to SOM among all fertilizer treatments, implying the strong influence of SOM on soil microbial activity. Murugan *et al.* (2014) investigated variations of catabolic function of different land use in Germany using MicroResp^{TM} method, showed significantly lower organic C content, biomass C and residue C in the monoculture maize compared to the grassland treatments, suggesting higher labile C present in the grassland systems promoted bacterial diversity.

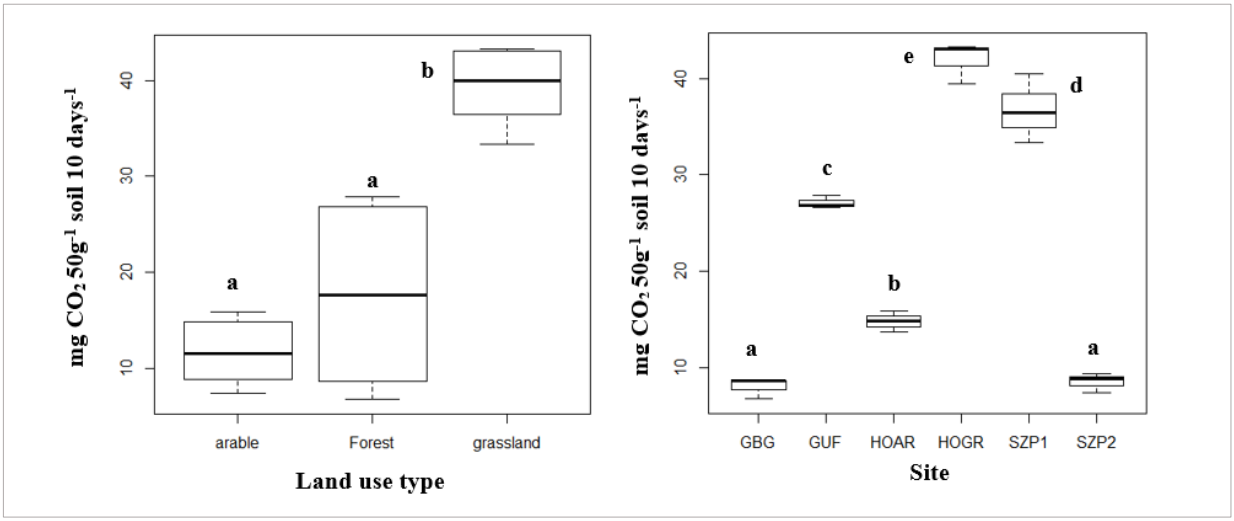


Figure 14. SMR across LUTs (left) and sites (right), with standard error bars. Different letters indicate significant differences (p < 0.05, n=3 for site and n= 6 for LUT). Abbreviations: Hort1 (HOGR), Hort2 (HOAR), Gödöllő botanical garden (GBG), Gödöllő university forest (GUF), Szárítópuszta 1 (SZP1), Szárítópuszta 2 (SZP2)

In this study, SMR was found positively correlated with MC (r = 0.67; p =0.002). Various studies documented that MC, among others, has important effect on soil microbial diversity and function (e.g. Conant *et al.*, 2004; Liu *et al*., 2009). Soil moisture influences other

Table 8. Pearson's correlation between physicochemical properties and SMR

Physicochemical properties	**SMR**	**p-value**
SOM	0.56	0.01*
pH(H_2O)	0.33	0.16
$CaCO_3$	-0.39	0.10
MC	0.66	0.002**
NH_4^+-N	0.06	0.08
NO_3^--N	0.08	0.74
Total N	0.08	0.73
AL-K_2O	0.57	0.01*
AL-P_2O_5	-0.34	0.15
Ca^{2+}	0.33	0.18
Mg^{2+}	0.60	0.007**
K^+	-0.38	0.11
Na^+	0.52	0.02*
CEC	0.36	0.13
BS	0.36	0.20
E_4/E_6	0.03	0.88

, **, *: Significant at the 0.05, 0.01, and 0.001 levels, respectively. Abbreviations: soil microbial respiration (SMR), soil organic matter (SOM), soil moisture content (MC), cation exchange capacity (CEC), base saturation (BS).*

physicochemical properties, such as, pH, O_2, CO_2, and redox potential which affects soil microbial communities and their activities (Barros *et al.*, 1995). Soil moisture can reduce soil respiration by lessening microbial contact with available substrate and dormancy and death of microorganisms at low soil water potentials (Conant *et al.*, 2004).

The significant effect of soil moisture in SMR was detected by Liu *et al*. (2009), who reported that decreased soil moisture caused significant reduction of SMR, suggesting soil water availability was more important than temperature in regulating soil microbial respiration in semiarid temperate grassland of China. Generally, soil respiration increases with soil moisture, and the ideal soil moisture to microbial activity is near field capacity (Liebig, 1996). In this study, the variation of SMR was greatly affected by MC, evidenced by strong positive correlation between these variables.

Among the base cations, Mg^{2+} showed a significant positive correlation with SMR (r = 0.61; p = 0.007). It is well known that Mg^{2+}, along with other base cations, is important soil nutrient that greatly influences the soil microbial population and their activity since it is required for microbial growth and protein synthesis (Rutgers *et al*., 2009). The finding of a strong positive correlation

between SMR and Mg^{2+} was also detected by Richter *et al.* (2018), who assessed the effect of diagnostic features, land use, and soil type on microbial biomass and microbial indices in Irish grassland.

This study also found a positive correlation of K_2O with SMR (r = 0.58; p = 0.01). Our finding contrasts with Mori (2018), who conducted an incubation experiment to examine the effects of K addition on SMR and soil microbial biomass in condition of sufficient labial C supply in China's tropical soils and found no significant effect of K addition on SMR. Various studies reported the significant effect of pH on SMR (Wakelin *et al.*, 2008; Andruschkewitsch *et al.*, 2014); however, this study did not find a statistically significant correlation of SMR with pH (r = 0.33; p = 0.1678). The neutral correlation between pH and SMR was also noted by Ebrahimi *et al.* (2019).

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

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

4.4.1. SMR and key soil physicochemical properties

The analytical data for soil properties are presented in Table 9. Briefly, BD varied from 1.10 g cm^{-3} in Vertisol to 1.47 g cm^{-3} in Luvisol (Appendix II). Generally, BD greater than 1.6 g cm^{-3} tends to restrict root growth (McKenzie *et al*., 2004), thus, the soils of the study area were not compacted to the extent of restricting root growth. Soils collected from Laelay Maichew (LMH) sites contained higher MC than soils from ATS site and it ranged from 3.05% in Nitisol to 10.07% in Cambisol. The higher MC in LMH sites were probably attributed to a high heavy clay content in these soils. SOC was highest in Nitisol (2.41%) and lowest in Cambisol (0.46%). The soil pH(H_2O) ranged from neutral (7.2) to slightly alkaline (7.9). While P_2O_5 content was highest in Nitisol, followed by Cambisol, Vertisol, and Luvisol, the K_2O content showed the opposite trend. Generally, all available forms of nitrogen and exchangeable bases were recorded highest in Nitisol. The high level of P_2O_5 in ATS-3 could be associated with the application of a large dose of diammonium phosphate (DAP: 18-46% N-P_2O_5) (Bekele *et al*., 2012). The rate of SMR ranged from 11.22 in Cambisol to 47.08 mg CO_2 50 g^{-1} soil $10days^{-1}$ in Nitisol. The CEC was highest in Vertisol, followed by Luvisol, Cambisol, and Nitisol. There was not any $CaCO_3$ detected in the soil samples.

The capacity of soil to provide soil functions can be predicted by the activity and abundance of microbial communities in relation to key soil properties, such as SOM, available nutrients, and pH (Richter *et al*., 2018). All three pedons in LMH sites (Luvisol, Cambisol, and Vertisol) had vertic

property on the surface soils and were similar to most physicochemical properties (Table 9). Hence, it would be expected that these soils have similar microbial communities and activities, thereby a similar rate of SMR. Consistently, the difference in mean SMR among soils in LMH sites was small, while the difference was big between soils in the ATS-3 site and in LMH sites. The high rate of SMR in Nitisol corresponded to the high amount of SOC and P_2O_5, which could explain the importance of these parameters for soil microbes' metabolic activity.

Table 9. Soil physicochemical properties and SMR across sites in Ethiopia

Soil properties	**Site**			
	LMH-1	**LMH-2**	**LMH-10**	**ATS-3**
SOC (%)	1.67(0.02)	0.46(0.01)	0.49(0.06)	2.41(0.06)
pH(H_2O)	7.93(0.08)	7.70(0.00)	7.86(0.03)	7.26(0.03)
MC (%)	5.36(0.79)	10.09(0.81)	6.66(0.43)	3.05(0.47)
P_2O_5 (mg kg^{-1})	15.67(0.04)	13.36(2.09)	13.36(2.09)	155.50(8.37)
K_2O (mg kg^{-1})	230.5(6.06)	287.5(21.07)	328.5(36.37)	184.5(11.25)
NH_4^+-N (mg kg^{-1})	1.15(0.11)	6.06(0.28)	7.23(1.50)	5.20(0.55)
NO_3^--N (mg kg^{-1})	1.95(0.31)	2.70(0.22)	2.50(0.11)	2.50(0.00)
E_4/E_6	1.57(0.02)	1.50(0.01)	1.48(0.01)	1.60(0.02)
Ca^{2+} (cmol kg^{-1})	20.94(0.74)	15.48(2.21)	18.93(0.28)	16.08(0.62)
Mg^{2+} (cmol kg^{-1})	4.10(0.15)	3.11(0.56)	3.85(0.06)	5.31(0.18)
K^+ (cmol kg^{-1})	0.45(0.03)	0.38(0.01)	0.44(0.00)	0.52(0.02)
Na^+ (cmol kg^{-1})	0.14(0.03)	0.04(0.03)	0.11(0.01)	0.30(0.03)
CEC (cmol kg^{-1})	46.71(0.17)	46.17(0.14)	47.25(0.82)	41.21(0.65)
BS (%)	54.88(1.84)	41.22 (6.25)	49.39(0.09)	54.20(2.96)
SMR (mg CO_2 $50g^{-1}$ soil $10days^{-1}$)	14.23(6.69)	11.22(3.89)	13.90(1.21)	47.08(6.69)

Values are means (standard errors). Abbreviations: soil organic carbon (SOC), soil moisture content (MC), cation exchange capacity (CEC), base saturation (BS), soil microbial respiration (SMR), Laelay Maichew (LMH), Atsbi Wenberta (ATS)

SOC is the primary energy source for microbes and considered one of the key soil attributes that greatly influences SMR (Creamer *et al*., 2014). Studying 117 different soils with a broad range of physicochemical properties in the Czech Republic, Hofman *et al*. (2004) concluded that SOC had a strong correlation with SMR and thus, greatly influence the overall soil microbial activity. The current study found that soils with lower E_4/E_6 ratio (more humified) had less SMR. This implies that the reduction of labile SOM could be one of the limiting factors for SMR in the study area (van Leeuwen et *al*., 2017).

One of the possible explanations for the high rate of SMR in Nitisol might be due to the very high content of P_2O_5 related to the continuous amendment of soils with diammonium phosphate (DAP). Currently, most studies concluded that beside C, soil microbial activity in tropical soils is highly limited by P availability. The change in P_2O_5 concentration in the soil causes a shift in the soil microbial communities' functional and metabolic potential, resulting in a change in decomposition rates. Phosphorous has a higher affinity to the sorption sites of mineral soils than labile C; thus, the addition of P would release organic matter bound to the sorption sites, which in turn stimulates soil microbial activity (Mori *et al*., 2018). Accordingly, Liu *et al*. (2013) reported that long-term P addition significantly increased soil respiration, suggesting that soil microbial activity enhanced by P addition. Conversely, Teklay *et al*. (2006) noted that the SMR pattern was more affected by N than P addition after the amendment of soils with glucose-C together with N and P at Wondo Genet in southern Ethiopia.

The pH has been shown to be one of the significant predictors for SMR (Creamer *et al*., 2015). Enzymes, involved in catabolism of carbon substrates, are pH sensitive (Richter *et al*., 2018). Soil pH influences the solubility of SOM and changes the rate of microbial carbon turnover. It also affects the availability and distribution of nutrients, which are essential for microbes to decompose SOM (Ebrahimi *et al*., 2019). This study found that soils collected from the ATS-3 site with lower pH (7.2) had four times higher SMR than soils with higher pH at LMH sites (7.7–7.9). Our result was confirmed by the finding of Creamer et al. (2016), who noted a decrement of basal respiration with pH. Among the base cations, Mg^{2+}, Na^{+}, and K^{+}, was in line with carbon utilization rate. A similar trend has been previously observed in Irish grassland by Richter *et al*. (2018). In general, this study highlighted that the variation of SMR in the study area was closely linked with the variation of P_2O_5, SOC, and Mg^{2+} (Figure 15).

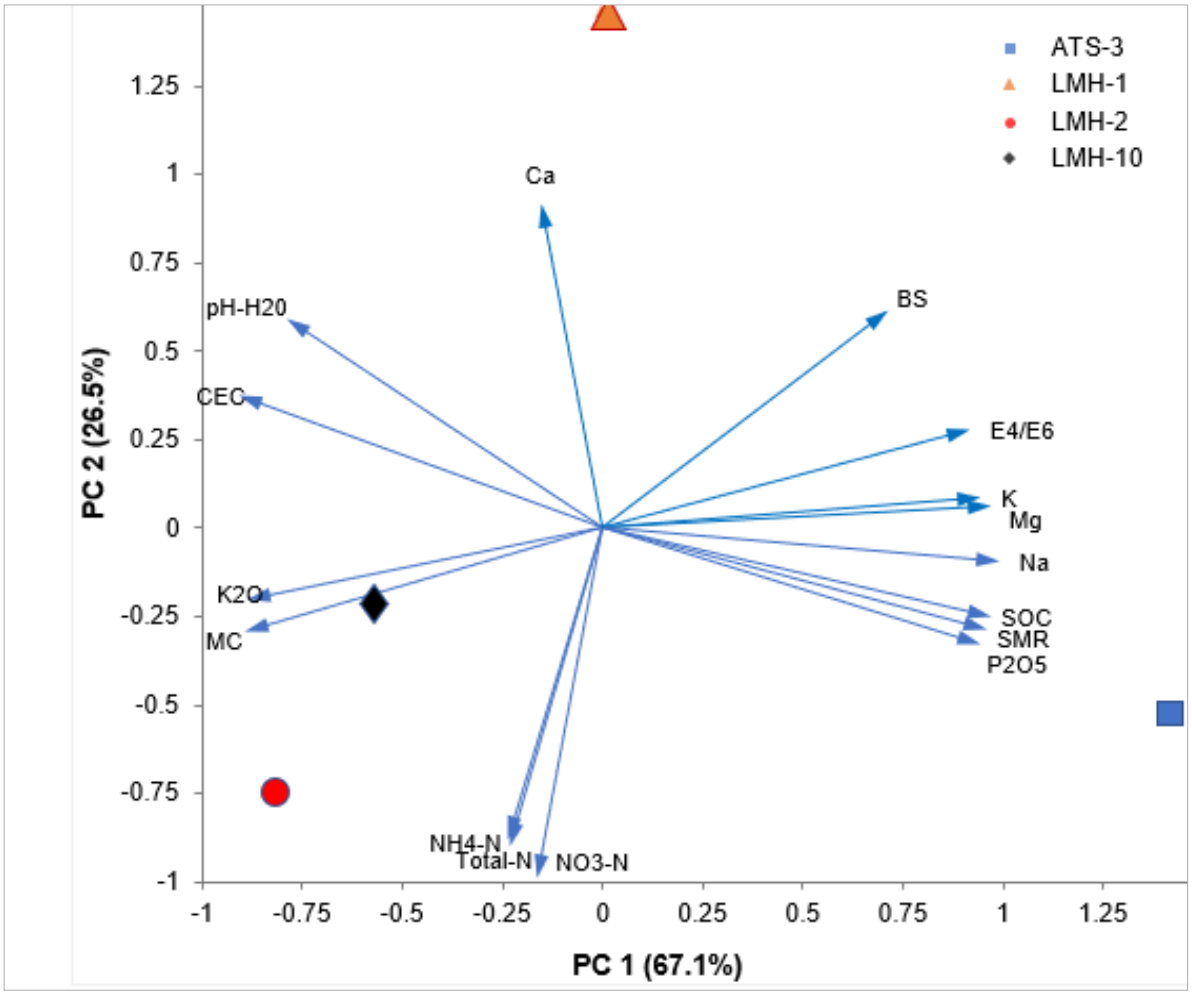


Figure 15. Principle component analysis between physicochemical properties and SMR. ATS-3 site clearly separated form LMH sites based on high content of P_2O_5, SOC, and Mg^{2+}. LMH-1 separated from other sites based on high content of Ca^{2+}. Abbreviations: soil organic carbon (SOC), soil moisture content (MC), cation exchange capacity (CEC), base saturation (BS), soil microbial respiration (SMR), Laelay Maichew (LMH), Atsbi Wenberta (ATS)

4.4.2. Bacteria community structure

Comparing the genus number according to the 400 bp amplicon sequencing results, Nitisol was the richest with 475 genera, Cambisol was the second with 389 genera, Luvisol showed 351 and Vertisol contained the lowest number 315. From these genera's operational taxonomic units (OTUs) (family or order in some cases), only 31 were showing relative abundance over 1%. The 31 genera showing abundance over 1% covered the 79% of all OTUs of Vertisol, 72% of Luvisol, 66% of Cambisol, and 58% of Nitisol (Table 10). Based on the heat tree figure, the Luvisol and Cambisol have a slight difference among the identified bacteria genera (Figure 16). The Vertisol showed a shift into the direction of *Actinobacteria – Pseudoarthrobacter* and *Gaiellales* family. In Nitisol samples, the *Proteobacteria – Alphaproteobacteria* class: *Sphingomonas* genus and

Rhizobiaceae family were prevalent. At the phylum level, there was only a slight difference among the investigated soils. The following phyla showed the greatest abundance in the samples: *Actinobacteria*, *Chloroflexi*, *Proteobacteria*, *Acidobacteria*, *Planctomycetes*, and *Gemmatimonadetes*. *Actinobacteria* was the most abundant phylum in all samples, and accounted (44%) in Vertisol, followed by Luvisol and Nitisol (27%).

Table 10. Genus level according the 400 bp reads over 1% abundance from the total reads. Taxonomy was made by the SILVA and GenBank database

					abundance in %			
Phylum	**Class**	**Order**	**Family**	**Genus**	**LMH-1**	**LMH-2**	**LMH-10**	**ATS-3**
Actinobacteria	Thermoleophilia	Gaiellales	Gaiellaceae	Gaiella	14.24	8.14	17.16	3.44
	Actinobacteria	Micrococcales	Micrococcaceae	Pseudarthrobacter	1.56	1.24	14.38	2.43
	Thermoleophilia	Solirubrobacterales	Solirubrobacteraceae	Solirubrobacter	2.26	3.35	3.52	9.48
	Rubrobacteria	Rubrobacterales	Rubrobacteriaceae	Rubrobacter	3.22	2.64	1.66	1.90
	Acidimicrobiia	Microtrichales	Ilumatobacteraceae	*Ilumatobacteraceae(f)*	2.27	0.83	3.03	0.84
	Actinobacteria	MB_A2_108	*MB_A2_108*	*Actinobacteria (c) MB_A2_108*	1.35	3.48	0.79	0.22
	Actinobacteria	Propionibacteriales	Nocardioidaceae	Nocardioides	0.62	0.52	2.10	2.58
	Acidimicrobiia	Microtrichales	*Microtrichales*	*Microtrichale (f)*	0.63	2.68	0.70	0.22
	Actinobacteria	Streptomycetales	Streptomycetaceae	Streptomyces	0.29	0.24	0.23	3.27
	Actinobacteria	Micromonosporales	Micromonosporaceae	Plantactinospora	0.45	1.01	0.26	0.75
	Actinobacteria	Frankiales	*Frankiaceae*	*Frankiaceae (f)*	0.34	1.18	0.27	0.07
	Actinobacteria	Corynebacteriales	Mycobacteriaceae	Mycobacterium	0.04	0.09	0.06	1.52
	Acidimicrobiia	*Acidimicrobiia*	*Acidimicrobiia*	*Acidimicrobiia(o)*	0.08	0.12	0.04	1.09
	Subtotal				27.35	25.50	44.20	27.82
Chloroflexi	Chloroflexia	Thermomicrobiales	Thermomicrobium	Thermomicrobium roseum	5.86	4.70	5.93	6.94
	Bacteria Ellin 6519	*Bacteria Ellin 6519*	*Bacteria Ellin 6519*	*Bacteria Ellin 6519*	8.88	4.60	6.04	0.84
	Chloroflexia	Kallotenuales	Kallotenuaceae	*Kallotenuaceae(f)*	2.13	1.02	1.31	0.51
	Chloroflexia	KD4_96	*KD4_96*	*Chloroflexia (c) KD4_96*	0.77	1.63	0.81	0.34
	Chloroflexia	Chloroflexales	Roseiflexaceae	*Roseiflexaceae(f)*	0.82	1.45	0.26	0.10
	Subtotal				18.46	13.40	14.36	8.73
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	2.07	1.46	2.12	6.07
	Alphaproteobacteria	Rhizobiales	Rhizobiaceae	*Rhizobiaceae(f)*	0.74	0.86	0.49	4.69
	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Microvirga	1.90	2.16	1.17	1.01
	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	*Xanthobacteraceae (f)*	1.21	1.05	0.69	1.98
	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Psychroglaciecola	1.23	0.97	0.62	0.12
	Betaproteobacteria	Burkholderiales	Burkholderialeceae	Ralstonia	1.04	0.51	1.20	0.14
	Alphaproteobacteria	Reyranellales	Reyranellaceae	Reyranella	0.35	0.29	0.24	1.31
	Alphaproteobacteria	Rhizobiales	Devosiaceae	*Devosiaceae(f)*	0.06	0.03	0.02	1.46
	Subtotal				8.60	7.33	6.56	16.78
Actinobacteria	Acidobacteria	Subgroup_6	Subgroup_6	Acidobacteria (c) Subgroup_6	5.76	6.42	5.02	1.24
	Blastocatellia	Blastocettales	Pyrinomonadaceae	Pyrinomonadaceae (f) RB41	2.28	3.60	1.53	0.14
	Subtotal				8.04	10.02	6.55	1.38
Planctomycetes	Planctomycetacia	Gemmatales	Gemmataceae	Gemmata	3.27	2.13	2.49	1.65
	Phycisphaerae	Tepidisphaerales	Planctomycetales	*Planctomycetales (f)*	1.51	3.88	0.84	1.03
	Subtotal				4.78	6.01	3.33	2.68
Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	*Gemmatimonadaceae(f)*	5.36	4.69	4.63	2.10
				Total %	**72.59**	**66.95**	**79.66**	**59.49**

(c) class level

(f) family level

(o) order level

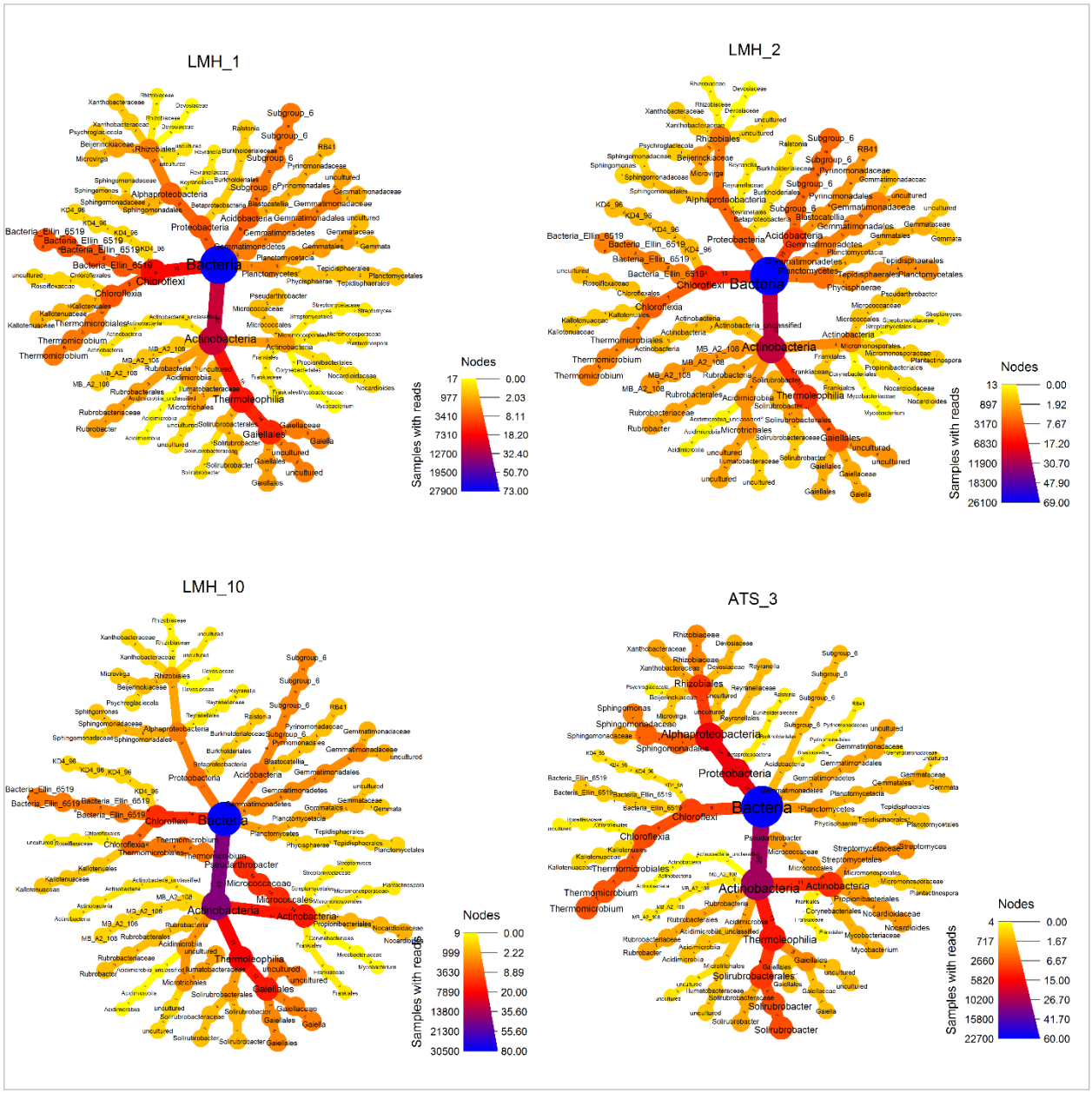


Figure 16. Heat tree map showing relative abundance of bacteria genera within the most abundant bacterial phyla

In the case of *Chlorflexi*, it was found abundantly in Luvisols (18%). While *Proteobacteria* showed the greatest abundance in Nitisol (15%) and the lowest in Vertisol (6%), the abundance of *Acidobacteria* decreased to the lowest (1.38%) in Nitisol. In general, these three phyla (*Actinobacteria*, *Chloroflexi*, *Proteobacteria*) gave the majority among the four soils: Vertisol: 65%, Luvisol: 54%, Nitisol: 51%, and Cambisol: 46%. The richest phylum, *Actinobacteria*, was represented by the following classes over 1% abundance: *Thermoleophilia*, *Actinobacteria*, *Acidimicrobiia*, and *Rubrobacter*. In Vertisol, from the total abundance of phylum *Actinobacteria* (49%), *Thermoleophilia* and *Actinobacteria* accounted for 20% and 18%, respectively. The *Thermoleophilia* class was represented by the *Gaiella* genus in a very high abundance Vertisol

(17%), Luvisol (14%), Cambisol (8%), and Nitisol (3%). The *Gaiella* genus has only one identified species yet (Albuquerque *et al*., 2011). The greatest abundance of class *Actinobacteria* was found in Vertisol. Within *Proteobacteria, Alphaproteobacteria* was the most abundant class, followed by *Betaproteobacteria*.

Among the *Chloroflexi* phylum, two classes were abundant over 1%: *Chloroflexi* and an uncultured class, *Bacterium Ellin-6519*. The LMH soils had a greater abundance (over 4%) of *Bacterium Ellin-6519* but its abundance was only 0.84% in the Nitisol. Interestingly among the genera of the *Chloroflexi* phylum, the *Thermomicrobium roseum* species was the most abundant (4–6%) in all soils. This species is an extremely thermophilic bacterium first isolated from an alkaline hot spring in Yellowstone National Park. It is an obligate aerobe and grows optimally at 70^{o} to 75^{o}C at a pH of 8.2 to 8.5 (Jackson *et al*., 1973).

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

The presence of the most common PGPB was investigated by the result of the amplicon sequencing data. The most common members of the PGPB are the *Azotobacter*, *Bacillus*, *Pseudomonas*, *Streptomyces* genera, and *Rhizobiaceae* family which are able to promote plant growth with different enzymatic activities such as N fixation, P mobilization, Indole Acetic Acid (IAA), and extracellular polysaccharide (EPS) production.

The predominant PGPB in the studied soil belongs to *Proteobacteria*. *Proteobacteria* are a phylum of Gram-negative bacteria commonly found in soil, involved in a wide range of functions such as carbon, nitrogen, and sulfur cycling (Aislabie and Deslippe, 2013). The *Alphaproteobacteria* was the most dominant class, occupied the highest abundance in Nitisol. The majority of sequences in the *Alphaproteobacteria* were affiliated with the order *Rhizobiales* (including *Rhizobiaceae, Beijerinckiaceae, Xanthobacteraceae, Devosiaceae* family), which could perform nitrogen fixation, organic matter decomposition, and plant growth promotion (Andrea *et al*., 2017). Studies reported that some *Proteobacteria*, e.g., *Pseudomonas* and *Beijerinckia*, can involve both in nitrification and

P-solubilization processes (e.g., Di Benedetto *et al*., 2017). Symbiotic bacteria play essential roles in the host plant's life by enhancing the nutrients uptake, suppressing diseases causing pathogens and pests, and enabling plants to adapt to various environmental stresses (Franche *et al*., 2009). Soil properties are known to condition the microbes' growth in soils (Andrea *et al*., 2017). Soil characteristics may influence specific lineages differently than they affect deeper taxonomic classifications. For instance, environmental effects on *Rhizobia* may differ from environmental effects on *Rhizobiales* as a whole (Kumar and Meena, 2019). Generally, pH is found to be a

primary driver, that influences the bacterial community at the phylum level (Fierer and Jackson, 2006). According to Rousk *et al*. (2010), *Proteobacteria* are increased in high pH levels, *Alphaproteobacteria* were most abundant in soils with high pH values. In our case, *Proteobacteria* phylum (containing *Rhizobiales*) was only present in 6–8% in the case of LMH soils with 7.7–7.9 pH; thus, the highest abundance was observed in ATS-3 soils (Nitisol) with 15% in 7.2 pH. Similarly, Lauber *et al*. (2009) discussed that the relative abundance of *Alphaproteobacteria* was not strongly influenced by pH. The SOM content was likely affecting members of this phylum since the abundance of *Alphaproteobacteria* was more pronounced in soils with high SOM concentration. A similar finding was noted by Tian *et al.* (2017), showed that the relative abundance of *Proteobacteria* increased with SOC.

The dominancy of *Actinobacteria* in all our soil samples may be related to their adaptation ability to typical semi-arid environment, similar to the study area (Rughöft *et al.,* 2016). *Actinobacteria* are Gram-positive bacteria that play vital roles in the cycling of organic compounds, production of antibiotic, and synthesis of growth hormones (Aislabie and Deslippe, 2013; Kumar and Meena, 2019). Among *Actinobacteria*, *Frankiaceae* was found in low abundance in the investigated soils. *Frankia* is the only genus in the family *Frankiaceae*, which can fix atmospheric nitrogen both in the free-living state and in association with several tree species. It is estimated that 70–100% of the host plant's nitrogen requirement is provided by nitrogen fixed by *Frankia* in root nodules (Kumar and Meena, 2019). Generally, *Actinobacteria* are sensitive to low pH and grow well in pH ranges between 6–8 (Aislabie and Deslippe, 2013), might also contribute to their frequent occurrence in our samples. Their abundance which increased with low organic carbon availability, has already been reported in other previous studies, e.g., Fierer *et al*. (2007). The NH_4^+-N concentration was the greatest 7.23 mg kg^{-1} in Vertisol; this could also affect the abundance of the *Actinobacteria* playing an important role in the carbon decomposition (Craine *et al*., 2007).

Among all IAA and phosphorus-mobilizing bacteria we identified, the majority belonged to *Bacillus*, *Paenibacillus,* and *Pseudomonas* but only in a very low abundance (0.1%). The very low abundance of phosphorus-mobilizing bacteria in this study contradicts the finding of Tsegaye *et al*. (2019). They investigated beneficial *Rhizobacteria* from teff rhizosphere samples collected during the seedling stage in Ethiopia and reported that 40.5% isolates were able to solubilize phosphate, of which, *Pseudomonas*, *Enterobacter*, and *Bacillus* were the dominant genera. The reason for very low abundance in our samples could be that our soils were bulk soils, not rhizosphere soils as in the mentioned study. Tropical soils are considered P-deficient because of their high acidity. Soil microbes help to release phosphorus that is only consumed in the soluble form such as monobasic (HPO_4^{2-}) and dibasic ($H_2PO_4^-$) phosphate (Kumar and Meena, 2019). The

use of PGPR strains with inherent potential for organic phosphorus mobilization offers a way to replace chemical phosphatic fertilizers, thereby minimizing environmental pollution.

Next to *Actinobacteria*, *Chloroflexi* were dominant in the soil samples, particularly in Luvisol. *Chloroflexi* phylum is one of the large phyla that comprises a large group of bacteria that acquire energy and fix CO_2 through photosynthesis, thus contributing to carbon dynamics (Aislabie and Deslippe, 2013). The previous findings that *Chloroflexi* prevails in nutrient-poor soils (Fierer *et al*., 2007), was also noted by this study as the abundance of *Chloroflexi* was lowest in Nitisol where the highest concentration of SOM was recorded.

Plant type has been suggested to be the dominant factor controlling the microbial community structure by driving changes in litter quality, pH, and soil moisture (Marschner *et al.*, 2002). Furthermore, agronomical practices, such as continuous tillage have been proven to influence the microbial communities and plant growth by affecting carbon and nutrient dynamics in the soil. Plant, soil, and management factors are interlinked and working in complex manner, exert a combined effect on soil microbes and thereby plant growth. In this study, the overall bacteria abundance was higher in wheat cropping than teff cropping fields. Teff, a warm-season annual cereal, is a major indigenous staple crop in Ethiopia. It is the most important crop in terms of cultivation area and production value in the country (Lee, 2018). Teff cultivation in Ethiopia needs high tillage frequencies as compared to other cereal crops. Further, teff cultivation on Vertisols requires several ploughings compared to Nitisols (Gebretsadik *et al.,* 2009). Based on a 2019 field experiment conducted by the ongoing project AFER (Agricultural Fertility and Environmental Resources 'plus), the teff grain yield was 2.24, 1.99, and 2.04 t ha^{-1} in LMH-1, LMH-2, and LMH-10, respectively, and the wheat crop yield in ATS-3 site was 2.13 t ha^{-1}. As of the report of CSA (2018), the national teff and wheat average grain yield in 2018 was 1.75 t ha^{-1} and 2.13t ha^{-1}, respectively, and this was lower than the regional teff (2.54 t ha^{-1}) and wheat (2.74 t ha^{-1}) yields. The low crop yield in the study area might be related to the low abundance of PGPB in the soil. Numerous studies confirmed that PGPB enhance plant growth and development by increasing nutrient availability and producing hormones that promote plant growth. For instance, Woyessa and Assefa (2011) reported that inoculation of teff crops with *Pseudomonas fluorescens* and *Bacillus subtilis* increased mean grain yield by 28% and 44%, respectively, that indicates the potential role of these bacteria in enhancing teff productivity However, these bacteria were in a very low abundance (0.02%) among the investigated samples.

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

PGPB abundance from the previously mentioned taxa. These results are marking the importance of adopting practices that encourage the elevation of SOM and P such as non-removal of crop residues from agricultural lands, application of manure, and most importantly, adaptation of P-mobilizing bacteria groups as PGPR fertilizer for better crop yield.

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

Among the investigated RSGs, only Luvisols and Vertisols were found both in Hungary and Ethiopia study sites, hence the comparison was only made between these RSGs. The results were based on soils samples collected from GBG (Luvisol), GUF (Luvisol), and VERP (Vertisol) sites (Hungary); LMH-1 (Luvisol) and LMH-10 (Vertisol) (Ethiopia). The metagenomic and amplicon sequencing data of the soil samples from Hungary presented in this research is not part of this PhD work. For comparison purpose, I used data from the work of Dalma Márton, a PhD student at Institute of Aquaculture and Environmental Safety, Hungarian University of Agriculture and Life Science. Soils from VERP, LMH-10, and LMH-1 were analyzed by amplicon sequencing method with the same exact protocol. However, soils from GUF were analyzed using metagenomic approach. Therefore, it should be noted that there could be a 10% or less difference between the results of metagenomic and amplicon sequencing data. The earthworms were only collected from sites in Hungary. Unfortunately, I was not able to do the earthworm sampling in Ethiopia due to time limitation and absence of species identification key for earthworms in Africa. The analytical results of major physicochemical are presented in Table 11

Table 11. Summary of soil properties among Luvisols and Vertisol of Hungary and Ethiopia

Property	**Unit**	**Hungary**		**Ethiopia**	
		Luvisol	**Vertisol**	**Luvisol**	**Vertisol**
SOM	(%)	4.85	2.56	1.15	0.85
pH(H_2O)		5.08	6.20	7.93	7.86
MC	(%)	21.58	26,64	5.36	6.66
Ca^{2+}	cmol kg^{-1}	5.75	23.20	20.94	18.93
Mg^{2+}	cmol kg^{-1}	0.92	6.04	4.10	3.85
K^{+}	cmol kg^{-1}	0.25	0.65	0.45	0.44
Na^{+}	cmol kg^{-1}	0.08	0.14	0.14	0.11
CEC	cmol kg^{-1}	33.63	36.1	46.71	47.25
BS	(%)	20.61	83.4	54.88	49.39

Abbreviations: soil organic matter (SOM), soil moisture content (MC), cation exchange capacity (CEC). Note: Values for Hungary Luvisol are the average of GBG and GUF sites

4.5.1. Variation of SMR among RSGs

The reason for the variability of biological component of the soil can be associate with the variability of soil physicochemical properties. Soil forming processes that contribute to the formation of different soil types have also influenced the biomass, composition, and activity of soil microbes (Maková *et al*., 2011). In addition, soil microbes greatly influenced by LUT (van Leeuwen, *et al*. 2017), soil management type (Castillo and Joergensen, 2001), and climatic factors (Zhang *et al*., 2017). The rate of SMR was relatively higher in both Luvisol and Vertisol of Hungary compared to Luvisol and Vertisol of Ethiopia, although the difference was not big. In both cases, SMR recorded higher in Luvisol than Vertisol (Figure 17). The relatively higher SMR in soils of Hungary probably related to the higher content of SOM and MC. The SOM and MC both were very low in soils from Ethiopia. Many studies reported that depletion of soil organic matter driven by severe erosion has been a major challenge of Ethiopian soils (e.g., Zelleke, 2010; Elias, 2016). Decomposition of SOM primary depend on the type of soil organic matter (Srivastava *et al*., 2017). SOM is a source of energy and nutrients required for microbial metabolic processes (Creamer *et al*., 2014). MC was 24% and 25% higher in Luvisol and Vertisol of Hungary compared to Luvisol and Vertisol of Ethiopia, respectively. Recently, it is found that SMR and carbon utilization during decomposition are interactively regulated by MC and temperature (Srivastava *et al*., 2017). Appropriate MC promote high rate of SOC decomposition. However, the impact of MC varies greatly depending on the temperature range and the level of SOC (Srivastava *et al*., 2017). Therefore, climatic factor also be a possible reason for low SMR in Ethiopia soils. Soils in drier (tropical) climatic regions expected to have low SOM and MC compared to wetter (temperate) climate (Creamer *et al*., 2016). Studies indicate that higher temperature in tropical regions enhance SOC decomposition leading to reduction of SOC in the soils (Sofi *et al*., 2016). Working in an Afromontane forest of south-eastern Ethiopia, Yohannes *et al.* (2011) found a strong correlation between SMR and MC, indicating the primary importance of soil moisture in controlling SMR.

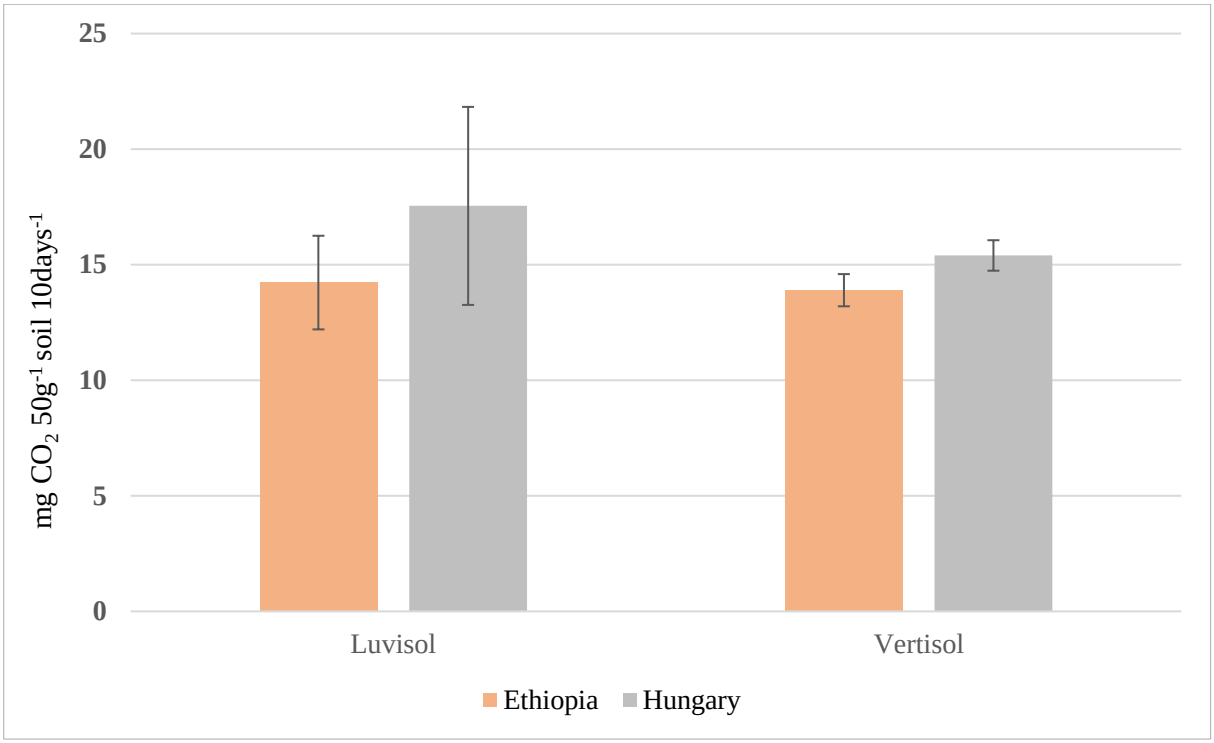


Figure 17. SMR among the two RSGs of Hungary and Ethiopia

Given that all soil samples from Ethiopia were taken from arable land whereas soils samples in Hungary collected from forest, it is particularly important to discuss the impact of LUT on SMR in these soils. Studies showed that SOM, nutrient, and MC in agricultural soils are low compared to forest and grassland soils (Celik, 2005). Moreover, compared to other soil types in Ethiopia, Vertisols and Luvisols are among the most intensively utilized soil types (Giday, 2015). The influence of soil type on SMR is difficult to predict, because it varies substantially with factors such as LUT, soil management, plant communities, and a range of other environmental factors. Therefore, multifactorial studies are required to understand how soil type, LUT, soil management, and climatic factors influence soil microbial activity for better soil management in the study area.

4.5.2. Patterns of bacterial community structure across RSGs

At phylum level, the A*ctinobacteria* was the most abundant phylum in LMH-10, LMH-1, and VERP soils, respectively. *Actinobacteria* highly dominated the soils from compared to soil from Hungary. Vertisol samples from Ethiopia (LMH-10) showed the greatest abundance of *Actinobacteria* (49%), more than twice of the abundance (23%) in Vertisol samples from Hungary (VERP). Similarly, the Luvisol from Ethiopia (LMH-1) showed three times higher *Actinobacteria* frequency compared to Luvisol from Hungary (GUF) (Figure 18). In Ethiopia soils, *Actinobacteria* phylum was more represented by *Thermoleophilia* class while in Hungary soils it was dominated by *Actinomycetia class*.

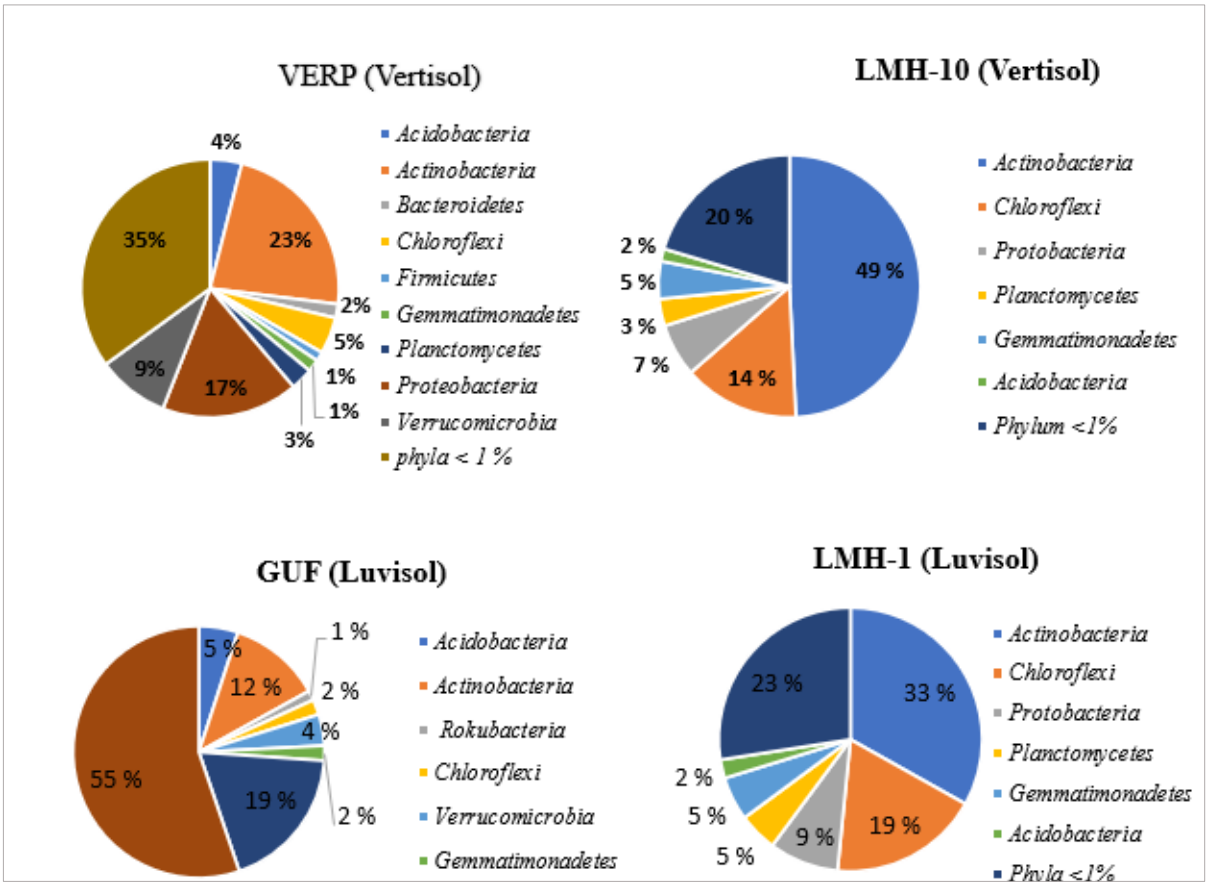


Figure 18. Distribution of bacteria phyla among RSGs of Hungary (left) and Ethiopia (right)

Within *Actinobacteria* phylum, genus *Gailla* highly characterized soils of Ethiopia, in Vertisol (17 %) and in Luvisol (14%). Its' abundance was low in soils from Hungary, although it dominated the VERP (Vertisol) sites with 4%. The genus *Gailla* is the only representative of family *Gaillaceae* and composed of a sole species isolated from a deep mineral water aquifer in Portugal. The optimum growth temperature is about 35–37ºC and the optimum pH for growth is between 6.5 and 7.5 (Albuquerque *et al*., 2011). Among the *Actinobacteria*, genus *Streptomyces* detected in higher than 1% abundance only in Luvisol of Hungary (2%) (Table 12). It was reported that some members of *Streptomyces* are acidophilic, and the low pH in GUF soils (pH= 5.5) could be the possible reason for the relatively higher frequency of *Streptomyces* in these soils. *Streptomyces* are one of the known IAA producing bacteria and biocontrol agents, serve as antibiotic and enzyme-producing microbes, and play important role in degradation of hydrocarbons (Bhatti *et al*., 2017).

Table 12. The relative abundance of bacteria genera exceeding 1 % among Luvisol and Vertisol of Hungary and Ethiopia. Taxonomy was made by the SILVA and GenBank database

					Abundance (%)			
					Hungary soils		Ethiopia Soils	
Phylum	**Class**	**Order**	**Family**	**Genus**	**VERP (Vertisol)**	**GUF (Luvisol)**	**LMH_10 (Vertisol)**	**LMH_1 (Luvisol)**
Actinobacteria	Actinomycetia	Streptomycetales	Streptomycetaceae	Streptomyces	< 1%	1.59	< 1%	< 1%
	Thermoleophilia	Solirubrobacterales	Solirubrobacteraceae	Solirubrobacter	1.99	1.11	3.52	2.26
	Thermoleophilia	Gaiellales	Gaiellaceae	Gaiella	3.82	< 1%	17.16	14.24
	Rubrobacteria	Rubrobacterales	Rubrobacteriaceae	*Rubrobacter*	1.30	< 1%	1.66	3.32
	Actinobacteria	Corynebacteriales	Mycobacteriaceae	Mycobacterium	1.11	< 1%	< 1%	< 1%
	MB-A2-108	MB-A2-108	MB-A2-108	*Actinobacteria (c) MB-A2-108*	1.11	< 1%	1.35	< 1%
	IM-CC-2625	IM-CC-2625	IM-CC-2625	Actinobacteria (c) IM-CC-2625	1.04	< 1%	< 1%	< 1%
	Actinomycetia	Propionibacteriales	Propionibacteriaceae	Microlunatus	1.03	< 1%	< 1%	< 1%
	Actinobacteria	Micrococcales	Micrococcaceae	Pseudarthrobacter	< 1%	< 1%	14.36	1.56
	Acidomicrobiia	Subgroup_6	Subgroup_6	*Acidobacteria (c) Subgroup_6*	< 1%	< 1%	5.02	5.76
	Acidimicrobiia	Microtrichales	Ilumatobacteraceae	*Ilumatobacteraceae(f)*	< 1%	< 1%	3.03	2.27
	Actinobacteria	Propionibacteriales	Nocardioidaceae	Nocardioides	< 1%	< 1%	2.10	0.62
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	< 1%	2.10	2.12	2.07
	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Microvirga	< 1%	< 1%	1.17	1.90
	Alphaproteobacteria	Rhizobiales	Xanthobacteraceae	*Xanthobacteraceae (f)*	1.96	< 1%	< 1%	1.21
	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	Psychroglaciecola	< 1%	< 1%	< 1%	1.23
	Betaproteobacteria	Burkholderiales	Burkholderialeceae	Ralstonia	< 1%	< 1%	1.20	1.04
	Alphaprotobacteria	Rhizobiales	Bradyrhizobiaceae	Bradyrhizobium	< 1%	3.57	< 1%	< 1%
	Alphaprotobacteria	Rhizobiales	Hyphomicrobiaceae	Rhodoplanes	< 1%	1.30	< 1%	< 1%
	SC-I-84	SC-I-84	SC-I-84	Betaproteobacteriales (c) SC-I-8	1.11	< 1%	< 1%	< 1%
Chloroflexi	Chloroflexia	Thermomicrobiales	Thermomicrobium	Thermomicrobium roseum	1.00	< 1%	5.93	5.86
	Bacteria Ellin 6519	*Bacteria Ellin 6519*	*Bacteria Ellin 6519*	*Bacteria Ellin 6519*	1.00	< 1%	6.04	8.88
	Chloroflexia	Kallotenuales	Kallotenuaceae	*Kallotenuaceae(f)*	< 1%	< 1%	1.31	2.13
	Chloroflexia	KD4_96	*KD4_96*	*Chloroflexia (c) KD4_96*	1.36	< 1%	< 1%	< 1%
Acidobacteria	Blastocatellia	Blastocettales	Pyrinomonadaceae	Pyrinomonadaceae *(f)* RB41	< 1%	< 1%	1.53	2.28
	Acidobacteriia	Acidobacteriales	Acidobacteriaceae	Acidobacteriaceae (f)	< 1%	1.34	< 1%	< 1%
	Subgroup_6	Subgroup_6	Subgroup_6	Acidobacteria (c) Subgroup_6	1.55	< 1%	< 1%	< 1%
	Acidobacteriia	Bryobacterales	Solibacteraceae	Sulfopaludibacter	< 1%	1.11	< 1%	< 1%
Planctomycetes	Planctomycetacia	Gemmatales	Gemmataceae	Gemmata	< 1%	< 1%	2.49	3.27
	Phycisphaerae	Tepidisphaerales	Planctomycetales	*Planctomycetales (f)*	1.59	< 1%	< 1%	1.51
Gemmatimonadetes	Gemmatimonadetes	Gemmatimonadales	Gemmatimonadaceae	*Gemmatimonadaceae(f)*	1.15*	< 1%	4.63	5.39
Verrucomicrobia	Spartobacteria	Chthoniobacterales	Chthoniobacteraceae	Candidatus Udaeobacter	7.42	< 1%	< 1%	< 1%

(c) means class level

(f) means family level

Investigating the bacterial diversity in soils of different Hungarian Karst area, Knáb *et al*. (2018) found the high abundance of *Streptomyces* in the studied soil samples.

The highest abundance of *Chloroflexi* was found in LMH-1 (18%) followed by LMH-10 (14%), VERP (4%), GUF (2%), respectively (Figure 18). These microbes can carry out anaerobic photosynthesis and remove electrons from hydrogen sulfide (Song *et al*., 2018). Recently, Ujvári *et al*. (2020) assessed the soil bacterial communities from juvenile maize plants of a long-term monoculture and a natural grassland in Hungary and found similar abundance (4%) of *Chloroflexi* in the studied soils. *Chloroflexi* from *bacteria Ellin 6519* and *Thermomicrobium roseum* genus presented in large number in soils from Ethiopian while it formed a minor part of the bacterial communities of samples from Hungary, mainly from representative of *bacteria KD4_96*.

Studies indicated that *Actinobacteria* and *Chloroflexi* phyla are more prevalent in arid and semi-arid environment (Rughöft *et al*., 2016) and in soils with poor nutrient content (Fierer *et al*., 2007). Particularly the low soil moisture and SOM availability in the Ethiopian soils could be the possible reasons for the high dominancy of *Actinobacteria* and *Chloroflexi* in Ethiopian soils. Comparing the bacterial community composition of pristine church forest soils and adjacent degraded agricultural soils in northwest highland of Ethiopia, Abebe *et al.* (2020) revealed that the *Actinobacteria* were more abundant in degraded soils which presents lower SOM (6%), and MC (11%) compared the pristine forest soils with high MC (22%) and SOM (9%).

The bacterial communities in GUF demonstrated an increase in the proportion of *Proteobacteria* phylum (19%) from the *Bradyrhizobium* genus, followed by VERP (17%) from family *Xanthobacteraceae*, confirming the findings of Ujvári *et al*. (2020) who found a close abundance of *Proteobacteria* (20.2–26.5%). The prevalent classes of *Proteobacteria* phylum in both Hungary and Ethiopia soils were *Alphaproteobacteria*, followed by *Betaproteobacteria. Gamma- and Delta- Proteobacteria* occurred in more than 1% abundance only in GUF. Predominant proportion of *Proteobacteria* in Ethiopia soils were belonging to genus *Siphingomonas* and family *Beijerinckiaceae*. Most members of *Proteobacteria* are fast growing r-strategist that can colonize plant root and can be abundant in soils with high nutrient availability (Fierer *et al*., 2007) and relatively high pH value (Rousk *et al*., 2010). The pH values recorded higher in soils from Ethiopia but the abundance of *Proteobacteria* recorded lower compared to soils from Hungary. Hence the distribution of *Proteobacteria* was more likely affected by SOM content than pH in the studied soils.

Acidobacteria phylum was more prevalent in GUF (5%) followed by VERP (4%), and LMH-10 and LMH-1 (2%). The distribution of sequences between members of *Acidobacteria* was quite different among samples from Hungary and Ethiopia. The *Acidobacteriaceae* family and *Subgroup_6* mostly characterized soils from Hungary, whereas family *Pyrinomonadaceae* represented the *Acidobacteria* communities of soils from Ethiopia in more than 1% abundance. A study by (Lin *et al*., 2019) indicated that the *Subgroup_6* prefers soils with pH below 5.5. Likewise, in Amazonian dark earth with high levels of SOM and nutrients and a pH about 5.0, Navarrete *et al*. (2010) found the high occurrence of *Subgroup_6*, justifying the dominancy of *Subgroup_6* in soil samples from Hungary with high SOM and low pH. The increment of *Acidobacteria* in GUF probably associated with the low pH, in agreement with previous study by Rousk *et al*. (2010). Likewise, Ujvári *et al*. (2020) revealed a much higher abundance of *Acidobacteria* (14.7–21.4%) in soil samples taken from Martonvásár, Hungary. *Acidobacteria* has the capacity to break down complex SOM and plant-derived polysaccharides (Song *et al*., 2018). Among members of *Actinobacteria*, *Sulfotelmatobacter*, a known sulfur removing bacteria, occurred in more than 1% abundance only in GUF soils (Table 12).

Phylum *Verrucomicrobia* were only detected in greater than 1% abundance in samples from Hungary (VERP: 9% and GUF: 4%). The recent findings of Carbonetto *et al*. (2014) revealed the higher abundance of *Verrucomicrobia* in uncultivated soils compared to cultivated soils, confirming the findings of higher abundance of *Verrucomicrobia* in forest soils of GUF and less utilized arable soils of VERP, compared to intensively utilized arable soils of Ethiopia. This is also in accordance with the findings of Ujvári *et al*. (2020) that showed the significantly higher abundance of *Verrucomicrobia* in the grassland than in the maize monoculture. The majority of sequences of *Verrucomicrobia* were affiliated to genus *Candidatus Udaeobacter*. *Gemmata* genus belonging to *Planctomycetes* phylum showed higher frequency in Ethiopia soils, while *Planctomycetales* family (also belonging to this phylum) was relatively abundant in Hungary soils with low abundance.

Phyla *Firmicutes* (2%), and Bacteroidetes (2%) were found in more than 1% abundance only in VERP soils. On the other hand, the *Gemmatimonade* phylum showed the opposite trend, more frequent in soils from Ethiopia, mostly from family *Gemmatimonadaceae*. The research findings of Tan *et al*. (2020) revealed that the occurrence of *Gemmatimonade* inversely proportional to moisture content, supporting the finding of high proportion of *Gemmatimonade* in soils from Ethiopia in this research. The *Rokubacteria* phyla detected only in GUF with 1% abundance.

Altogether, this study found the greatest difference in the abundance of *Gailla, Ellin 6519, Thermomicrobium roseum, Gemmatimonadaceae, Acidobacteria Subgroup 6* among the

investigated Hungary and Ethiopia soils. The first four bacterial groups found in great abundance in soil from Ethiopia and the last in soils from Hungary. The Luvisol and Vertisol of Ethiopia greatly differed in *Pseudarthrobacter* abundance, which was higher in Vertisol. The biggest difference among Vertisol and Luvisol of Hungary was with respect to a high occurrence of genus *Candidatus Udasobacter* belonging to *Verrucomicrobia* phylum in Vertisol samples. The distribution of bacterial communities of the studied soils seemed to depend on geographical location rather than soil type. Soils from the same country showed similar composition of bacterial communities irrespective of differences in soil type (RSG). Site specific soil properties, particularly SOM and soil moisture content might be the key soil properties that discriminate soils among the two countries. However, it should be noted that most of the OTUs from both Hungary and Ethiopia soils developed in unclassified or uncultured closely related sequences, which could be detected only in higher taxonomic levels. Moreover, the influence of MC on the bacterial community of the investigated soils should be interpreted in caution as it was only based on one field measurement. The average soil moisture data during the vegetation period could provide a closer interpretation.

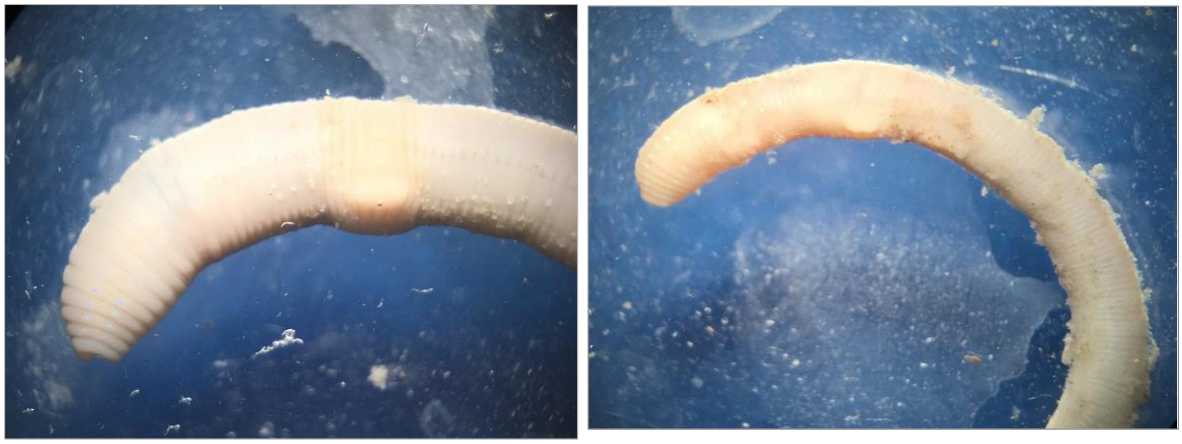
4.5.3. Earthworm communities among Vertisols and Luvisols of Hungary

All measured earthworm parameters were significantly higher in Vertisol than Luvisol. On average, earthworm abundance ranged from 40.00 to 880.00 ind. m^{-2} and earthworm biomass from 5.65 to 92.51 g m^{-2} in Luvisols and Vertisols, respectively. In total five earthworm species were identified in Vertisols, however the Luvisol sites harbor only one species (Table 12). The most common earthworm species was *Aporrectodea rosea* (Figure 19). Despite the higher SOM in Luvisol sites (Table 11), the earthworm communities were lower than Vertisol site. This could probable due to low pH in Gödöllő forest sites where Luvisols are located. The pH value of Verpelét site was 6.20 whereas the GUF and GBG were 4.07 and 5.49, respectively. Studies documented that acidic soils are unsuitable for earthworm inhabitation (Moore *et al*., 2013). The difference in pH could also explained the significant difference in earthworm communities between the two Luvisol sites (GUF, GBG).

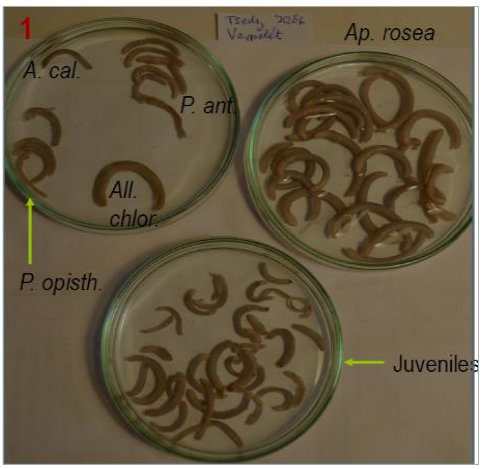
Table 12. Earthworm abundance, biomass, and species across Luvisols and Vertisols of Hungary

Site	RSG	Earthworm abundance (ind m^{-2})			Total biomass (g m^{-2})	Earthworm species
		Adult	Juvenile	Total		
VERP	Vertisol	389	491	880	92.51	37 *Aporrectodea rosea* 17 *Proctodrilus opisthoductus* 16 *Proctodrilus antipai* 2 *Aporrectodea caliginosa* 1 *Allolobophora chlorotica*
GUF	Luvisol	6	37	43	6.84	1 *Aporrectodea caliginosa*
GBG	Luvisol	0	11	11	1.00	

The values are means of three replicates. Abbreviations: Reference soil group (RSG), Gödöllő botanical garden (GBG), Gödöllő university forest (GUF), Verpelét (VERP)



a) b)



c)

Figure 19. a) Aporrectodea rosea, b) Proctodrilus opisthoductus Dish, c) Earthworm samples in Petridish (Photo by Dr. Barbara Simon, 2020)

4.6. New scientific results

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

2. For the first time, the bacterial community structure and trend of SMR of dominant agricultural soil types of Ethiopia (Nitisols, Vertisols, Luvisols, and Cambisols) were determined. From the results, I have confirmed that:
   i. the highest bacterial genera and SMR was found in Nitisol where high content of P_2O_5, SOC, and exchangeable Mg^2 recorded.
   ii. Genus *Gailla* (from phylum *Actinobacteria), Thermomicrobium roseum* and unclassified *Bacterium Ellin-6519* (from phylum *Chloroflexi),* and order *Rhizobiales* (from phylum *Proteobacteria)* were the most abundant bacterial groups in the studied soils
   iii. From the identified PGPB, *Streptomyces*, *Sphingomonas*, *Ralstonia* (genus), *Rhizobiaceae*, *Frankiaceae*, *Devosiaceae* (family) were relatively abundant
   iv. The abundance of essential PGPB was low (<1%), indicating the importance of adopting proper soil managements that encourage the elevation of SOM and P availability to enhance the important agrobacterial community for better crop yield.
3. Bacterial community structure and trend of SMR were compared among similar RSGs of Hungary and Ethiopia. Soils originated from some geographical location with different RSGs showed more similar bacterial composition than soils with the same RSG but belong to a different country. Site specific soil attributes particularly SOM and MC found to be the key soil properties that discriminate soils among the two countries.

5. CONCLUSIONS AND RECOMMENDATIONS

The results of soils from Hungary showed that SMR was greatly influenced by soil type whereby mollic soils (Chernozems and Phaeozems) had significantly higher microbial respiration compared to non-mollic soils (Luvisols and Arenosols). There was differential effect of LUT on soil physicochemical properties and SMR that varied depending on the site and the time of sampling. However, in all cases, grassland soils showed higher SMR compared to forest and arable lands, respectively. Among the physicochemical variables, NO_3^--N, total N, K_2O, SOM, were significantly influenced by LUT. Overall, there was substantially lower concentration of available nitrogen and SOM in arable soils compared to forest and grassland soils, suggesting agricultural practices in the study area induced reduction of SOM/SOC and available N. Soil depth had significant influence on NH_4^+-N, NO_3^--N, and total N), CEC, and Mg^{2+}. Soil microbial respiration greatly differed among the two soil depths (0–10 and 10–25cm) with a higher rate of SMR in surface soils compared to subsurface soils. Generally, the variation of SMR was closely linked with the variation of available N, SOM, MC, K_2O, Ca^{2+}, and Mg^{2+}, implying that these soil properties were key drivers of SMR in the study area.

The earthworm biomass and abundance varied significantly across soil and LUTs, however, explicit correlations with any of soil property measured was not observed. Generally, compared to arable and forest sites, grassland sites were more favorable for earthworm communities. Agricultural practices related to tillage may had a profound effect on earthworm communities than soil properties.

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

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

5.1. Recommendations

- This study did not clearly outline the extent to which soil inherited properties and LUT contributed to the total effect on the investigated biological parameters. More

research that investigates the two variables separately is suggested to come up with a better conclusion.

- The influence of plant communities particularly litter quality on soil microbial and earthworm communities is difficult to disentangle from the influence of soil characteristics. Hence, further research is needed to investigate vegetation effect on bacterial abundance, SMR, and earthworm communities to obtain a net effect of soil properties and LUT in the study area.
- Temporal and/or spatial replicate for each soil type (in case of soils from Ethiopia) need to be done to get a comprehensive view of agrobacterial community structure and SMR profile of the investigated soil types.
- To get inclusive view on selected biological characteristics of major RSGs of Hungary and Ethiopia, soils that occur exclusively in the arid and semi-arid environments of Ethiopia (Gypsisols and Solonchaks), and important RSGs of Hungary such as salt affected (Solonetz) and groundwater-affected (Gleysols) soils need to be investigated.

6. SUMMARY

Following the 1992 Earth Summit in Rio de Janeiro, soil biodiversity has been recognized globally as a crucial player in guaranteeing the functioning of soil and a provider of several ecosystem services essential for human well-being. The microbial fraction of the soil is an essential component of soil fertility as soil microbes play key roles in soil aggregate formation, nutrient cycling, humification, and degradation of pollutants. Soil fauna, such as earthworms have huge impacts on SOM and nutrient cycling and infiltration and distribution of water in the soil. Soil properties and LUTs are prominent ecological factors that govern the composition and activity of soil microbial and earthworm communities. Understanding the influence of soil properties and LUTs in soil biodiversity is crucial for sustainable land management, thereby, to protect and regenerate the soil ability to deliver ecosystem services vital to human well-being.

This study includes two parts; in the first part, soil sampling was carried out from nine soil profiles in Hungary. I employed basal respiration and hand sorting methods as described by ISO guidelines to investigate the patterns of soil microbial respiration and earthworm (abundance, biomass, and species richness) across various soil types and three LUTs (forest, grassland, and arable land). Soil samples were taken from the top 25 cm and along 0–10 and 10–25cm depths of the soil (in case of Szárhalmi forest, Károly-magaslat, and Csobánc sites).

SMR was significantly higher in mollic soils compared to non-mollic soils, with highest values in Chernozem soils and lowest in Arenosols SMR greatly differed among the two soil depths ($p = 0.0005$) with a higher rate of SMR in surface soils compared to subsurface soils. Among the investigated soil physicochemical parameters, N, NO_3^--N, NH_4^+-N, SOM, MC, K_2O, Ca^{2+}, and Mg^{2+}, were significantly positively correlated with SMR. The influence of LUT on soil physicochemical and SMR varied depending on the site and the time of soil sampling. Generally, grassland soils had higher microbial activity compared to forest and arable land. The results showed differential effects of soil depth on chemical properties where available nitrogen (NH_4^+-N, NO_3^--N, and total N), CEC, and Mg^{2+} were strongly influenced by soil depth. Most of the investigated soil properties demonstrated significant difference across LUTs, among which, NO_3^--N, total N, and K_2O were profoundly affected by LUTs ($p \leq 0.001$). On the other hand, CEC, MC, and Na^+ did not change significantly among the LUTs ($p \geq 0.05$). Overall, arable soils showed the lowest concentration of SOM and available nitrogen but highest content of P_2O_5 and $CaCO_3$, although there were few exceptions.

The earthworm biomass and abundance varied significantly across soil and LUTs, however, explicit correlations with any of soil property measured was not observed. A total of five

earthworm species were identified, i.e., *Aporrectodea caliginosa*, *Octolasion lacteum*, *Aporrectodea rosea*, *Proctodrilus opisthoductus*, and *Aporrectodea georgii*. Earthworm abundance, biomass, and species richness tend to be highest in grassland and lowest in arable land.

The second part of the research involved soil samples collected from selected four major reference soil groups (Luvisols, Cambisols, Vertisols, and Nitisols) in the Tigray Regional State of Ethiopia. I employed amplicon sequencing and basal respiration methods to investigate the bacterial community structure and rate of microbial respiration, respectively. SMR was significantly higher in Nitisol sample with a high amount of P_2O_5, soil organic carbon (SOC), and exchangeable Mg^{2+}. Amplicon sequencing results (400 bp/ OTU reads) revealed that the bacterial community was dominated by *Actinobacteria*, *Chloroflexi*, *Proteobacteria*, *Planctomycetes*, *Gemmatimonadetes*, and *Acidobacteria* phyla. *Actinobacteria* was the most abundant phylum in all samples but most prevalent in Vertisol. The highest ratio of *Proteobacteria* was in the Nitisol while that of *Chlorflex*i was in Luvisols. *Thermoleophilia* and *Actinobacteria* classes were the most abundant classes within the *Actinobacteria* phyla. The *Chloroflexi* phyla was dominated by class *Chloflexi* and an uncultured class: *Bacterium Ellin-6519*. Within *Proteobacteria*, *Alphaproteobacteria* was the most abundant class, followed by *Betaproteobacteria*. The abundance of well-known plant growth promoting bacteria (PGPB) was very low. Altogether Nitisol showed the highest abundance of the known PGPB with 15%, represented over 1% abundance by the following taxa: *Streptomyces*, *Sphingomonas*, *Ralstonia* genus, *Rhizobiaceae*, *Frankiaceae*, *Devosiaceae* family. The other soils had a total of only 4% PGPB abundance from the previously mentioned taxa.

SMR was compared among similar RSG of Hungary and Ethiopia, and it was higher in Vertisols and Luvisol of Hungary compared to Vertisol and Luvisol of Ethiopia. The distribution of major bacterial phyla showed differences among similar RSGs of Hungary and Ethiopia. Irrespective of differences in soil type, soils from Ethiopia showed the higher abundance of *Actinobacteria* and *Chloroflexi* phyla whereas, the *Proteobacteria* and *Acidobacteria* dominated the Hungary soils. SOM and MC could be key soil attributes that differentiated the SMR and bacteria communities among Hungary and Ethiopia soils.

From the first part of the research, it can be concluded that available N, SOM, MC, K_2O, Ca^{2+}, and Mg^{2+} were key soil properties that drive the variation of SMR in the study area. Agricultural activities induced reduction of SOM and available nitrogen in the study area. The earthworm communities of Hungary were more influenced by agricultural activities related to tillage than the inherited soil properties. The results of the soil samples from Ethiopia indicated that P_2O_5, SOC, and Mg^{2+} predominantly explained the variability of bacterial community structure and pattern of SMR in those sites. The well-known agroecosystem bacteria members (PGPB) were very low in

abundance, marking the importance of the future use of manure or possible agrobacteria fertilizer to increase the fertility and nutrient uptake of the crop plants in the region. Based on comparison results among similar RSGs of Hungary and Ethiopia, the bacterial composition found to be more influenced by geographical origin than soil type.

7. ÖSSZEFOGLALÁS

Az 1992-ben Rio de Janeiro-ban tartott Föld Csúcstalálkozót követően a talaj biológiai sokféleségét világszerte a talaj megfelelő működésének biztosításában kulcsfontosságú szereplőnek, valamint az emberi jólét szempontjából elengedhetetlen ökoszisztéma-szolgáltatónak tekintik. A talaj mikrobiális része a talajtermékenység nélkülözhetetlen eleme, mivel a talaj mikroorganizmusai kulcsszerepet játszanak az aggregátumok képzésében, a tápanyagok körforgásában, a humifikációban és a szennyező anyagok lebontásában. A talajfaunának, például a földigilisztáknak, nagy hatása van a talaj szerves anyagára, a tápanyagok körforgására, valamint a víz beszivárgására és eloszlására. A talaj tulajdonságai és a területhasználati módok kiemelkedő ökológiai tényezők, amelyek szabályozzák a talaj mikrobiális és földigiliszta közösségének összetételét és aktivitását. A talaj tulajdonságainak és a területhasználati módoknak a talaj biológiai sokféleségében gyakorolt hatásának megértése kulcsfontosságú a fenntartható területgazdálkodás szempontjából, vagyis talajainkat védeni és regenerálni kell annak érdekében, hogy az emberi jólét szempontjából nélkülözhetetlen ökoszisztéma szolgáltatásokat biztosítani tudja.

Ez a tanulmány két részből áll; az első részben a talajmintavételezésre kilenc talajszelvényből került sor Magyarországon. Az ISO Szabványok által leírt módon mikrobiális talajlégzés és kézi válogatás módszerét alkalmaztam a talaj mikrobiális légzésének és a giliszta paraméterek (abundancia, biomassza és fajgazdagság) vizsgálatára a különböző talajtípusok és három területhasználat (erdő, gyep és szántóföld) esetén. A talajmintákat a talaj felső 25 cm-ről, valamint 0–10 és 10–25 cm-es mélységből vettük (Szárhalmi erdő, Károly-magaslat és Csobánc).

Az mikrobiális talajlégzés szignifikánsan magasabb volt az ún. „mollic", mint a „nem mollic" típusú talajokban, a legmagasabb értékek a Chernozem, míg a legalacsonyabbak az Arenosol talajokban voltak. Az mikrobiális talajlégzés nagymértékben különbözött a két talajmélység között ($p=0,0005$), a felszíni talajokban magasabb volt az aránya a felszín alatti talajréteghez képest. A vizsgált talajfizikai-kémiai paraméterek közül az N, NO_3^--N, NH_4^+-N, szerves anyag, talajnedvesség, K_2O, Ca^{2+} és Mg^{2+} szignifikánsan pozitívan korrelált a mikrobiális talajlégzéssel. A területhasználat hatása a talaj fizikokémiai tulajdonságaira, valamint a mikrobiális talajlégzésre a vizsgált területtől és a talajmintavétel idejétől függően változott.

A gyep vegetációjú talajok általában magasabb mikrobiális aktivitással rendelkeztek az erdő és a szántó területekhez képest. Az eredmények a talaj mélységének kémiai tulajdonságokra gyakorolt különbségeit mutatták, ahol a felvehető nitrogént (NH_4^+-N, NO_3^--N és összes N), a kationcsere-kapacitást és a Mg^{2+} tartalmat erősen befolyásolta a talaj mélysége. A legtöbb vizsgált

talajtulajdonság szignifikáns különbséget mutatott a területhasználati módok között, amelyek közül az NO_3^--N, az összes N és a K_2O-t nagymértékben függtek a területhasználattól ($p \leq 0{,}001$). Másrészt a kationcsere-kapacitás, a talajnedvesség és a Na^+ tartalom nem változott szignifikánsan a területhasználati módok között ($p \geq 0{,}05$). Összességében elmondható, hogy a szántóföldi talajok adták a legalacsonyabb szerves anyag és felvehető nitrogén, de a legmagasabb P_2O_5 és $CaCO_3$ tartalmat, bár akadt néhány kivétel.

A földigiliszták biomasszája és egyedszáma a talajtípusok és a területhasználati módok között szignifikánsan eltért, azonban a mért talaj tulajdonságok egyikével sem mutatott egyértelmű összefüggést. Összesen öt földigiliszta fajt azonosítottunk (*Aporrectodea caliginosa, Octolasion lacteum, Aporrectodea rosea, Proctodrilus opisthoductus* és *Aporrectodea georgii)*. A földigiliszták biomasszája és egyedszáma a füves területeken a legmagasabb, míg a szántókon a legalacsonyabb volt.

A kutatás második része a kiválasztott négy fő talaj referencia csoportból (Luvisols, Cambisols, Vertisols és Nitisols) Etiópiában (Tigray Regional State) gyűjtött talajminták vizsgálatát mutatja be. A baktériumok közösségszerkezetét, valamint a mikrobiális talajlégzést vizsgáltam amplicon szekvenálás és respirációs módszerekkel. Az mikrobiális talajlégzés szignifikánsan magasabb volt a Nitisol talajban, amelyben nagy mennyiségű P_2O_5, szerves szén és kicserélhető Mg^{2+} volt. Az amplicon szekvenálás eredménye (400 bp / OTU olvasás) feltárta, hogy a baktériumok közösségében az *Actinobacteria*, a *Chloroflexi*, a *Proteobacteria*, a *Planctomycetes*, a *Gemmatimonadetes* és az *Acidobacteria* törzsek domináltak. Az *Aktinobaktérium* volt a leggyakoribb törzs az összes mintában, de a legelterjedtebb a Vertisolban volt. A *Proteobaktérium*-ok legnagyobb arányban a Nitisolban, míg a *Chlorflexi* a Luvisolban volt. A *Termoleophilia* és az *Actinobacteria* osztályok voltak a leggyakoribbak az *Actinobacteria* törzsön belül. A *Chloroflexi* törzset a *Chloflexi* osztály és egy ki nem tenyésztett osztály dominálta: *Bacterium Ellin-6519*. A *Proteobaktérium*-okon belül az *Alfaproteobaktérium*-ok voltak a legelterjedtebbek, melyeket a *Betaproteobaktérium*-ok követtek. A jól ismert növénynövekedést serkentő baktériumok (PGPB) abundanciája nagyon alacsony volt. Összességében a Nitisol mutatta az ismert PGPB legnagyobb mennyiségét, 15%-kal, amelyet a következő taxonok reprezentáltak több, mint 1%-ban: *Streptomyces, Sphingomonas, Ralstonia* génusz, *Rhizobiaceae, Frankiaceae, Devosiaceae* család. A többi talajban a növénynövekedést elősegítő baktériumok aránya a fent említett taxonokból csak 4% volt.

A mikrobiális talajlégzést összehasonlítottuk a magyarországi és az etiópiai hasonló talaj referencia csoportok között, és a magyarországi Vertisolban és Luvisolban magasabb volt, mint az etiópiai Vertisol és Luvisol esetében. A fő baktérium törzsek eloszlása különbségeket mutatott

Magyarország és Etiópia hasonló talaj referencia csoportjai között. A talajtípus különbségeitől függetlenül az etiópiai talajoknál nagyobb volt az *Actinobacterium* és a *Chloroflexi* törzsek előfordulási gyakorisága, míg a magyarországi talajokban a *Proteobaktérium*-ok és az *Acidobacterium*-ok domináltak. A talaj szerves anyaga és a talaj- nedvesség tartalma kulcsfontosságú tulajdonságok lehetnek, amelyek révén a mikrobiális talajlégzés és a baktériumok közösségei különböznek Magyarország és Etiópia talajai között.

A kutatás első részéből arra lehet következtetni, hogy a felvehető N, szerves anyag, talajnedvesség, K_2O, Ca^{2+} és Mg^{2+} kulcsfontosságú talajtulajdonságok voltak, amelyek a mikrobiális talajlégzés eltéréseit meghatározták a vizsgált területen. A mezőgazdasági tevékenységek a talaj szerves anyag és a felvehető nitrogén csökkenését okozták a vizsgált területen. Magyarország földigiliszta közösségeire a talajműveléssel kapcsolatos mezőgazdasági tevékenységek nagyobb hatással voltak, mint az öröklött talajtulajdonságok. Az etióp talajminták eredményei azt mutatták, hogy elsősorban a P_2O_5, szerves szén és Mg^{2+} tartalom magyarázta a baktériumközösség szerkezetének és a mikrobiális talajlégzés változékonyságát ezeken a területeken. A jól ismert PGPB nagyon alacsony mennyiségben voltak, jelezve, hogy az istállótrágya vagy az agrobaktérium-készítmények jövőbeni felhasználásának fontosságát a régióban a növények tápanyagfelvételének növelése érdekében. Magyarország és Etiópia hasonló talaj referencia csoportjai között végzett összehasonlás alapján megállapíthatjuk, hogy a baktériumok összetételét jobban befolyásolta a földrajzi eredet, mint a talajtípus.

8. RELATED PUBLICATIONS

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

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

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

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

Tsedekech Gebremeskel, Lubangakene Denish, Barbara Simon, Erika Micheli (2018): The role of Earthworms in Soil Carbon Dynamics. A review. 3rd International Young Researcher Scientific Conference- "Sustainable Regional Development Challenges of Space & Society in the 21st Century", April 26, Szent István University, Gödöllő, Hungary. ISBN 978-963-269-730-7

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

Weldmichael, T.G., Denish, L., Simon, B., Szegi, T., Michéli, E. (2019): Soil Moisture Content is Governed by a Combination of Soil Texture and Soil Organic Matter in Selected Soils of

Hungary, 21st Century Water Management in the Intersection of Sciences, March 22, Szarvas, p 366–372Hungary. ISBN 978-963-269-808-3

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

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

Weldmichael, T.G., Szegi, T., Denish, L., Gangwar, R. K., Michéli, E., Simon, B. (2020): Significant Influence of Land Use Type on Earthworm Communities but Not on Soil Microbial Respiration in Selected Soils of Hungary. ICSBB 2020: 22nd International Conference on Soil Biology and Biochemistry, p 945, March,12–13, London, United Kingdom. (Abstract)

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APPENDIX I. Soil profiles description and classification of soils from Hungary

Location: University Farm, Józsefmajor (JM1), Profile No.1.

GPS Coordinates: N 47º 41' 29.16", E 19º 36' 34.14"
Altitude: 149 m
Topography: Almost flat (A)
Slope: 1%

Parent material: loess
Temperature regime: Mesic
Soil moisture regime: Ustic
Land use: Annual field cropping (AA)

Soil profile description

Ap Dark grayish brown (10YR4/2), very dark grayish brown (10YR3/2) moist clay loam. Weak platy on top, fine angular blocky. Compacted, when dry. Slightly sticky when moist. No effervescence. Gradual smooth boundary.

A Very dark grayish brown (10YR3/2) very dark gray (10YR3/1) moist clay loam. Fine and medium granular structure. Slightly sticky and plastic when moist. Abundant wormholes, casts and crotovinas. No effervescence. Gradual smooth boundary.

Bw Brown (10YR4/3), dark brown (10YR3/3) moist, soft. Fine and medium granular structure. Slightly sticky and plastic when moist. Abundant wormholes, casts and crotovinas. No effervescence. Clear boundary.

BCk1 Light brownish gray (10YR6/2), grayish brown (10YR5/2) moist. Weak fine to medium subangular blocky structure. Calcium carbonate accumulation in forms of fine powdery coatings, and fillings of pores and root channels. Common crotovinas. Common loess snails. Strong effervescence. Gradual smooth boundary.

BCk2 Pale brown (10YR6/3), brown (10YR5/3) moist. Medium subangular. Non sticky or slightly plastic. Calcium carbonate accumulation in forms of fine powdery coatings, fillings of pores and root channels, and hard nodules (up to 1,5 cm). Soft. Strong effervescence.

2Bbk (Underlain by reddish paleosol)

Analytical data

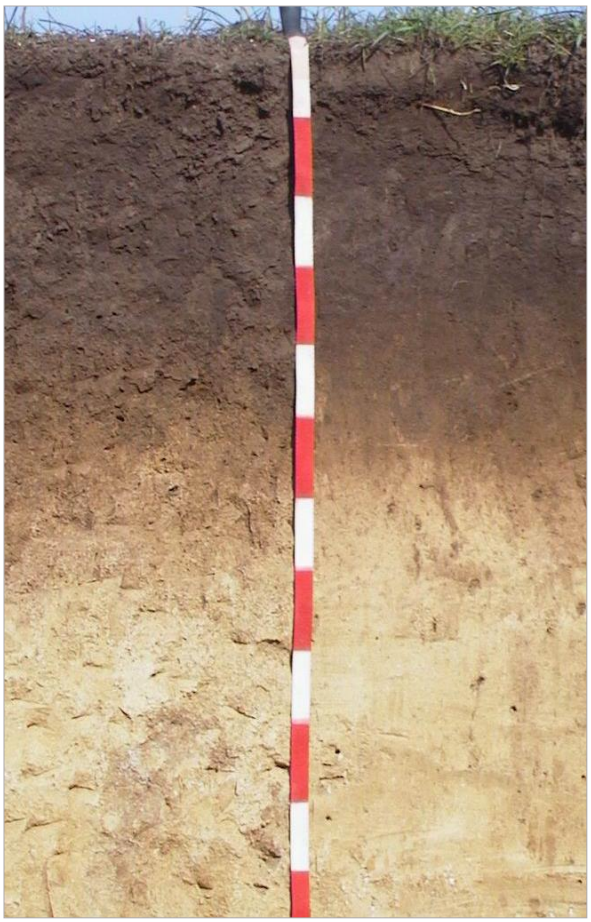
Genetic horizon	Depth cm	pH H_2O	OM %	$CaCO_3$ %	CEC cmol kg^{-1}	BS %	Sand % 2-0.05 mm	Clay % <0.002 mm	Texture (FAO)	BD g cm^{-3}
Ap	0-40	6,1	2,2	0,0	30	55	37	36	CL	1,4
A	40-60	6,9	1,6	0,0	29	60	36	37	CL	1,3
Bw	60-90	7,1	0,5	0,0	19	92	37	33	CL	1,3
BCk1	90-130	8,1	0,4	26,0	14	100	41	34	CL	1,2
BCk2	130-160	8,2	0,2	31,0	13	100	43	32	CL	1,3
2Bbk	160-	7,9	0,1	22,0	32	100	19	42	CL	

Soil type: Vermic Calcic **Chernozem** (Aric, Loamic, Pachic, Raptic)

Location: University Farm, Józsefmajor (JM2), Profile No.2.

GPS Coordinates: N 47° 41' 43.8", E 19° 36' 31.14"
Altitude : 139 m
Topography: Gently undulating (G)
Slope: 3%

Parent material: loess
Temperature regime: Mesic
Soil moisture regime: Ustic
Land use: Annual field cropping (AA)



Soil profile description

Ap Dark grayish brown (10YR4/2), very dark grayish brown (10YR3/2) moist, clay loam. Slightly sticky, very compacted, hard when dry. The upper part of the horizon is platy, the lower part has strong medium prismatic structure. No effervescence. Clears smooth boundary.

AB Brown (10YR4/3), dark brown (10YR3/3) moist, clay loam. Fine subangular structure. Slightly sticky and plastic Abundant wormholes and casts. Organic matter coatings on peds. No effervescence. Gradual wavy boundary.

BCk Light brownish gray (10YR6/2), grayish brown (10YR5/2) moist, clay loam. Weak medium subangular blocky structure. Friable, slightly sticky, slightly plastic. Calcium carbonate accumulation in forms of fine powdery coatings, and fillings of pores and root channels. Common wormholes, casts, crotovinas. Few broken loess snails. Gradual smooth boundary.

2CBk Pale brown (10YR6/3), brown (10YR5/3) moist, loam. Weak medium subangular blocky structure. Friable, nonsticky. Calcium carbonate accumulation in forms of fine powdery coatings, fillings of pores and root channels and hard nodules (~1cm). Soft, friable.

Analytical data

Genetic horizon	Depth	pH	OM	$CaCO_3$	CEC	BS	% Sand	% Clay	Texture	BD
	cm	H_2O	%	%	cmol kg^{-1}	%	2-0.05 mm	<0.002 mm	(FAO)	g cm^{-3}
Ap	0-30	6.1	1.5	0	28	84	34	32	CL	1.5
AB	30-50	6.9	1.6	0	30	89	35	37	CL	1.3
BCk	50-90	7.13	0.5	9	18	100	34	31.9	CL	1.3
2CBk	90-150	7.9	0.4	25	14	100	49	29	L	1.2

Soil type: Calcic **Chernozem** (Aric, Loamic, Raptic)

Location: University Farm, Józsefmajor (JM3), Profile No.3

GPS Coordinates: N 47º 41' 56.1", E 19º 36' 33.54"
Altitude : 126 m
Topography: Almost flat (A)
Slope: 1%

Parent material: local alluvial and colluvial sediments
Temperature regime: Mesic
Soil moisture regime: Udic
Land use: grass

Soil profile description

A1 Dark greyish brown (10YR4/3), very dark greyish brown (10YR3/2) moist, silty clay loam. Loose fine to medium subangular blocky structure. Slightly sticky and plastic. Abundant coarse partially decayed roots. No effervescence. Diffuse boundary.

A2 Dark greyish brown (10YR4/2), very dark greyish brown (10YR3/2) moist clay loam. Loose. Fine to medium subangular blocky structure. Slightly sticky and plastic. Few coarse partially decayed roots. Common wormholes and casts. Slight effervescence in places. Gradual smooth boundary.

2A Very dark greyish brown (10YR3/2) very dark grey (10YR3/1) moist, clay loam. Medium subangular blocky structure. Slightly sticky and plastic. Abundant wormholes, casts and crotovinas. Few fine secondary carbonate concretions. Clear wavy boundary (abrupt in color) with crotovinas extending into the lower horizons.

3Akl Thin layer of grey (10YR6/1), (10YR5/1) moist. Friable when wet. Hard and cemented when dry. Strong effervescence. Positive reaction with L-L dipyridyl.

4Ckl Pale brown (10YR6/3), brown (10YR5/3) moist loessy material. Weak medium subangular blocky structure. Abundant crotovinas (10-25 cm in ϕ). Strong effervescence. Positive reaction with L-L dipyridyl.

Analytical data

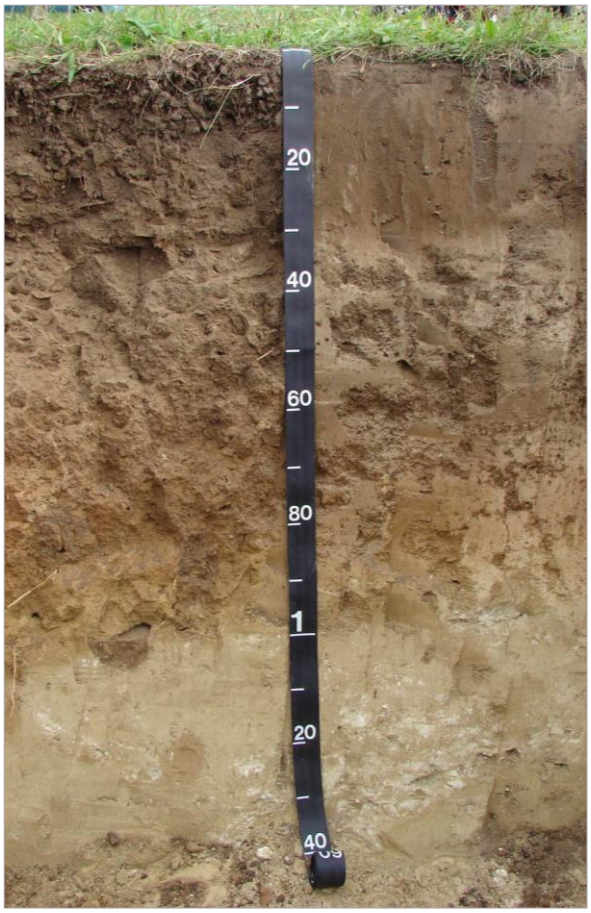
Genetic horizon	Depth	pH	OM	$CaCO_3$	CEC	BS	% Sand	% Clay	Texture	BD
	cm	H_2O	%	%	cmol kg^{-1}	%	2-0.02 mm	<0.002 mm	(FAO)	g cm^{-3}
A1	0-20	7.6	2.5	2.0	40.0	100	16.8	30.9	SiCL	1.19
A2	20-40	7,6	2,1	2,5	36,5	100	18,7	32,3	SiCL	1.23
2A	40-80	7.8	1.6	3.0	34.2	100	23.3	35.1	CL	1.43
3Akl	80-120	8.1	0.97	8.0	29.2	100	22.8	35.7	CL	1.51
4Ckl	120-130	8.6	0.47	28.0	28.3	100	35.0	44.5	C	1.48
5Ckl	130-	8.6	0.23	17.0	15.8	100	38	29.0	L	1.51

Soil type: Vermic Gleyic Calcic **Chernozem** (Amphiloamic, Bathyclayic, Pachic, Raptic)

Location: Szárítópuszta (SZP1), Profile No. 4.

GPS Coordinates: N 47°34'47.80", E 19°22'29.94"
Altitude : 222 m
Topography: Almost flat (A)
Slope: 1%

Parent material: Eolian loess and sand
Temperature regime: Mesic
Soil moisture regime: Ustic
Land use: grass



Soil profile description

Ap Brown (10YR 4/3), dark brown (10YR 3/3) moist, sandy loam. Moderate, fine to medium subangular blocky structure, slightly hard when dry. Very few fine, rounded gravel and charcoal. Very few fine roots. Few medium, very few fine pores. Common earthworm and ant channels. No effervescence. Gradual smooth boundary.

A2 Brown (10YR 5/3), dark yellowish brown (10YR 4/2) moist, sandy loam. Weak to moderate, fine and medium subangular blocky structure. Soft when dry. Very few fine, rounded gravel. Common, medium pores. Very fine few roots. Many earthworm and ant channels. No effervescence. Clear smooth boundary.

AB Yellowish brown (10YR 5/4), dark yellowish brown (10YR 4/4) moist, sandy loam. Moderate medium angular blocky primary, fine to medium subangular blocky secondray structure. Very few fine roots. Common medium, very few coarse pores. Many earthworm channels. No effervescence. Clear smooth boundary.

Bt Yellowish brown (10YR 5/6), dark yellowish brown (10YR 4/6) moist, loam. Moderate medium prismatic primary, fine to medium subangular blocks structure. Abundant distinct clay coating on pedfaces and in voids. Very hard when dry. Few medium pores. Many earthworm channels. Very few infilled large burrows. No effervescence. Abrupt wavy boundary.

Ck Pale yellow (2,5 Y 8/2), light yellowish brown (2,5Y 6/4) moist, sandy loam. Strong effervescence. $CaCO_3$ accumulation in the forms of small concretions, in root channels and coatings.

Analytical data

Genetic horizon	Depth	pH	OM	$CaCO_3$	CEC	BS	% Sand	% Clay	Texture	BD
	cm	H_2O	%	%	cmol kg^{-1}	%	2-0.02 mm	<0.002 mm	(FAO)	g cm^{-3}
Ap	0-30	7,2	2,9	0	21.7	100	70	9	SL	1,23
A2	30-50	7,2	1,1	0	19.2	100	62	13	SL	1,31
AB	50-65	7,1	0,2	0	19.8	100	53	17	SL	1,44
Bt	65-90	7,1	0,1	0	23.4	100	50	22	L	1,52
Ck	90-	7,5	-	27	10.6	100	52	12	SL	1,25

Soil type: Calcic Chernic **Phaeozem** (Loamic, Pachic)

Location: Szárítópuszta (SZP2), Profile No. 5.

GPS Coordinates: N 47°34'40.87", E 19°22'53.66"E
Altitude: 232 m
Topography: Almost flat (A)
Slope: 1%

Parent material: Eolian loess and sand
Temperature regime: Mesic
Soil moisture regime: Ustic
Land use: Annual field cropping (AA)

Soil profile description

Apk (0-25 cm)	Munsell moist (10YR 4/5), sand, weak granular structure. Clear wavy boundary. Strongly calcareous, calcium carbonate coatings.
Ck (25-45 cm)	Munsell moist (10YR 6/6), sand, subangular structure, Clear wavy boundary. Strongly calcareous, calcium carbonate coatings.
2Ck (45-70 cm)	Munsell moist (10YR 6/4), sand, Sub- granular structure. Abrupt wavy boundary. Strongly calcareous, Calcium carbonate coatings.
3Ck (70-80 cm)	Munsell moist (10YR 6/5), sand, moderate angular structure. Abrupt wavy boundary. Extremely calcareous, calcium carbonate coatings
4Ck (80-120 cm)	Munsell moist (10YR 5/4), sand, sub-angular structure, clear wavy boundary, Strongly calcareous, calcium carbonate coatings
5Ck (20-150 cm)	Munsell moist (10YR 6/5), sand, moderate angular structure. Extremely calcareous, calcium carbonate coatings.

Analytical data

Genetic horizon	depth cm	pH H_2O	OC %	CEC cmol kg^{-1}	BS %	% Sand 2-0.05 mm	% Clay <0.002 mm	Texture (FAO)	BD g cm^{-3}
Apk	0-25	8.4	0,90	10.2	100	81.1	6.3	S	1.23
Ck	25-45	8.4	0.77	8.2	100	88.3	5.7	S	1.24
2Ck	45-70	8.6	0.77	7.1	100	90.3	3.1	S	1.22
3Ck	70-80	8.7	0.45	7.2	100	88.3	5.7	S	1.23
4Ck	80-120	8.7	0.19	6.3	100	82.1	8.2	S	1.24
5Ck	120-150	8.5	0.22	3.4	100	87.6	2.5	S	1.13

Soil type: Eutrict **Arenosol** (Aeolic, Aric, Ochric, Raptic)

Location: Gödöllő Botanical Garden (GBG), Profile No. 6

GPS coordinates: N 47°35'40.75", E 19°22'17.28"
Landform: Gently sloping
Topography: plateau
Land use: botanical garden

Temperature regime: Mesic
Moisture regime: Ustic
Parent material: Pleistocene loess

Soil profile description

A (5-18 cm)	Munsell moist (10YR 4/3), sandy loam, subangular blocky structure, abrupt smooth boundary. No effervescence
EBt (18-38 cm)	Munsell moist (10YR 5/4), sandy loam, angular blocky structure. clear wavy boundary. clay coatings. No effervescence
Bt (38-65 cm)	Munsell moist (10YR 5/3), sandy clay loam, prismatic structure. gradual wavy boundary. No effervescence, clay coatings
Btss (65-110 cm)	Munsell moist (10YR 4/4), clay, prismatic structure, clear wavy boundary, No effervescence, clay coatings
5Ck (20-150 cm)	Munsell moist (10YR 6/6), sandy loam, moderate angular structure. Abrupt wavy boundary. Extremely calcareous, calcium carbonate coatings.

Analytical data

Genetic horizon	depth cm	pH H_2O	OC %	$CaCO_3$ %	CEC cmol kg^{-1}	BS %	% Sand 2-0.05 mm	% Clay <0.002 mm	Texture (FAO)	BD g cm^{-3}
O	0-5									
A	5-18	5.5	1.9	0	22.3	49.2	62.5	11.1	SL	1.3
EBt	18-38	5.6	1.0	0	16.2	55.4	61.3	17.3	SL	1.4
Bt	38-65	5.8	0.8	0	20.8	62.5	53.6	29.2	SCL	1.4
Btss	65-110	5.8	0.2	0	26.1	78.4	37.5	45.1	C	1.6
Ck	110-130	8.5	0.1	23	11.5	100	57.3	15.0	SL	1.2

Soil type: Haplic **Luvisol** (Amphiloamic, Bathyclayic, cutanic, Humic, Protovertic)

Location: Gödöllő University forest, Profile No. 7

GPS Coordinates: N 47°35'39.83", E 19°22'24.75"
Landform: Gently sloping
Topography: plateau
Land use: forestry
Temperature regime: Mesic
Temperature regime: Mesic
Moisture regime: Ustic
Parental material: Pleistocene loess

Soil profile description

A (3-10 cm)	Munsell moist (10YR 3/3), sandy loam, subangular blocky structure, Clear wavy boundary. No effervescence
AB (10-35 cm)	Munsell moist (10YR 4/3), sandy loam, subangular blocky structure. clear wavy boundary. clay coatings. No effervescence
Bw (35-45 cm)	Munsell moist (10YR 4/4), sandy clay loam, subangular blocky structure. gradual wavy boundary. No effervescence
Bt (45-75 cm)	Munsell moist (10YR 4/6), sandy clay loam, prismatic structure, gradual irregular boundary, No effervescence, clay coatings
BC (78-85 cm)	Munsell moist (10YR 5/4), sandy loam, subangular Structure, gradual irregular boundary, No effervescence.
2CK (85-120)	Munsell moist (10YR 6/4), loamy sand, subangular structure. Extremely calcareous, calcium carbonate coatings.

Analytical data

Genetic horizon	depth cm	pH H_2O	OC %	$CaCO_3$ %	CEC cmol kg^{-1}	BS %	% Sand 2-0.05 mm	% Clay <0.002 mm	Texture (FAO)	BD g cm^{-3}
O	0-3									
A	3-10	4.7	2.2	0	20.5	46.5	72.9	9.7	SL	1.3
AB	10-35	4.5	0.4	0	10.4	49.5	75.5	14.9	SL	1.42
Bw	35-45	4.8	0.3	0	12	59.3	72.1	18.2	SL	1.43
Bt	45-75	5.2	0.2	0	15.2	67	68.2	26.8	SCL	1.56
BC	75-85	6.5	0.1	2	8.8	82	70.3	19.8	SL	1.28
2Ck	85-120	8.1	0.1	12	8.3	92	85.1	3.8	LS	1.21

Soil type: Calcic **Luvisol** (Amphiloamic, Endoarenic, Cutanic, Humic, Raptic)

Location: Sopron, Szárhalom-forest (SZHE) Profile No. 8

GPS coordinates: N 47°41'41", E 16°50'31"
Altitude: 253 m
Vegetation: Forest (*Carpinus spp.*)

Relief: Sloping land (S), Middle slope (MS)
Slope gradient: Sloping (5-10°)
Parent material: loess

Soil profile description

A1 (0-12 cm)	Dark brown (10YR 3/2) moist, loam. Moderate granular structure. Clear smooth boundary. No effervescence.
A2 (12-30 cm)	Brown (10YR 3/3) moist, loam. Moderate angular blocky structure. No effervescence. Clear smooth boundary.
2A (30-40 cm)	Dark yellowish brown (10YR 3/4) moist, loam. Moderate angular blocky structure. No effervescence. Clear smooth boundary.
Bk (40-60 cm)	Brown (7,5YR 4/3) moist, loam. Moderate angular blocky structure. Moderately calcareous. Clear smooth boundary.
2Ck (60-75 cm)	Light olive brown (2,5Y 5/4) moist, loam. Extremely calcareous. Gradual smooth boundary.
2Ck2 (75-120 cm)	Yellowish brown (10YR 5/4) moist, loam. Extremely calcareous. Disperse powdery lime. Abrupt smooth boundary.
3Ck (120-160 cm)	Pale brown (2,5Y 7/3) moist, loam. Extremely calcareous. Disperse powdery lime.

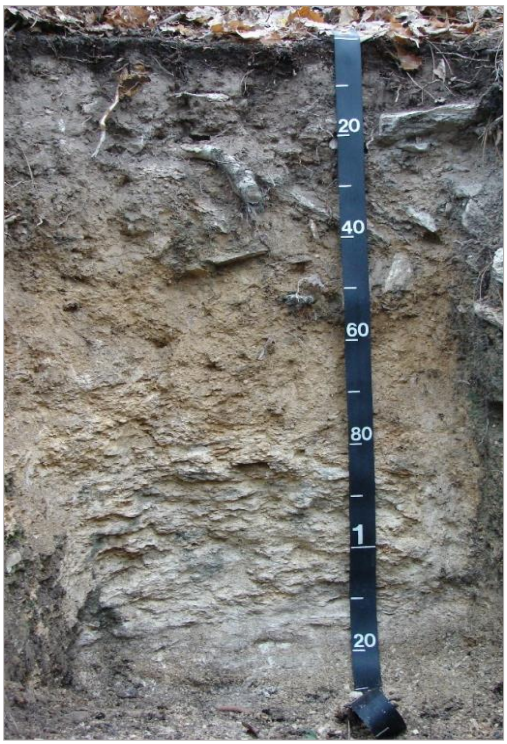
Analytical data

Genetic horizon	**Depth cm**	**pH H_2O**	**pH KCl**	**$CaCO_3$ %**	**OM %**	**Coarse sand 2.0 - 02 mm**	**Fine sand 0.2-0.02 mm**	**Silt 0.05-0.002 mm**	**Clay <0.002 mm**	**Texture (FAO)**
A1	0-12	7.3	6.7	5.59	3.6	3	46	48	3	L
A2	12-30	8.0	7.1	13.46-	3.6	2	47	32	19	L
2A	30-40	6.5	5.6	-	1.69	2	45	42	11	L
2Bk	40-60	7.3	6.7	4	0.91	4	43	48	5	L
2Ck	60-75	8.0	7.3	34	1.36	5	46	44	5	L
2Ck2	75-120	8.2	7.7	43	0.86	11	48	36	5	L
3Ck	120-160	8.4	8.0	64	0.22	16	65	16	3	S

Soil type (WRB. 2015): Greyzemic Calcic **Kastanozem** (Loamic, Colluvic)

Location: Sopron, Károly-magaslat (KAMG), Profile No. 9

GPS coordinates:	N 47° 39' 49.14", E 17° 33' 41.1"
Altitude:	370 m
Land use:	Plantation forestry (FP)
Vegetation:	*Quercus petrea, Fagus silvatica, Larix decidua, Picea abies*
Parent material:	Phyllite (MA3) and colluvium (UC1)



Soil profile description

A (0 – 5 cm)	Sandy loam, very dark grey (10YR 3/1), black (10YR 2/1) moist, very few, fine, (slightly) weathered rock fragments, strongly acid pH, weak fine/very fine subangular blocky structure. Fine and medium sized, common roots. Clear smooth boundary.
E (5 – 35 cm)	Sandy loam, light gray (10YR 7/2), dark grayish brown (10YR 4/2) moist, strongly acid pH, weak, thin subangular blocky structure. Many, coarse gravel and stones, slightly weathered phyllite. Few medium, and very few coarse roots. Gradual wavy boundary.
Bt/R (35 – 70 cm)	Sandy loam, very pale brown (10YR 7/4), yellowish brown (10YR 5/4) moist, acid pH, weak to moderate, medium subangular blocky structure. Few medium, and very few coarse roots. Few faint clay skins between and on surface of coarse fragments. Abundant, medium, and coarse slightly weathered phyllite (few stones). Gradual wavy boundary.
R (75 cm –)	Geological strata of slightly weathered phyllite

Analytical data

Genetic horizon	depth cm	pH H_2O	pH KCl	OC %	$CaCO_3$ %	CEC cmol kg^{-1}	BS %	∑Sand 2000-63 µm	∑ Silt 63-2 µm	∑ Clay < 2 µm	Texture (FAO)	BD g cm^{-3}
A	0-5	3.5	2.8	34.62	0	43.35	47.04	74.3	21.0	4.7	SL	1.3
E	5-35	3.5	3.1	3.55	0	39.56	6.36	61.8	28.0	10.2	SL	1.4
Bt/R	35-70	4.4	3.8	1.28	0	12.88	5.87	63.8	24.4	11.8	SL	1.4
R	70-	-	-	-	-	-	-	-	-	-	-	-

Soil type (WRB,2015) : Albic Endoskeletic **Alisol (**Cutanic, Humic, Hyperdystric, Loamic, Bathyleptic**.**

Location: Csobánc (CSOB), Profile No. 10

GPS coordinates : N 46°52'18.50", E 17°30'16.35"
Altitude: 370 m
Vegetation: grassy
Land use: pasture
Temperature regime: Mesic
Moisture regime: Ustic

Relief: Upland
Slope gradient: Flat (1-2%)
Parent material: basalt

Soil profile description

A1i (0-5 cm)	Black (10YR 2/1) moist, sandy clay loam texture. Granular structure. Dense root system. No effervescence. Diffuse transition.
A2(5-25 cm)	Very dark brown (10YR 2/2) moist, sandy clay loam texture. Granular structure. Dense root system. No effervescence. Diffuse transition
A/D (25-45 cm)	No effervescence. Diffuse transition.
C/D (45 -)	

Analytic data

Genetic horizon	Depth (cm)	pH H_2O	OC (%)	$CaCO_3$ (%)	CEC cmol kg^{-1}	B %	% Sand 2-0.05 mm	% Clay <0.002 mm	Texture (FAO)	BD g cm^{-3}
A1	0-5	6.4	8,8	0	23	55%	52	26	SCL	0,9
A2	5-25	6.5	7,2	0	26	57%	54	25	SCL	1,2
A/D	25-45			0						
C/D	45-			0						

Soil type (WRB, 2015): Sceletic **Phaeozem** (Loamic)

APPENDIX II. Soil profiles descriptions and classification of soils from Ethiopia

Location: Laelay Maichew, Axum (LMH-1), Profile No. 1

GPS coordinates: N 14° 06' 49", E 38° 46' 34" Land use: Crop agriculture (*Eragrostis tef (Zuccagni) Trotter*)

Altitude: 2074m Slope: 2%

Topography: Gently sloping Landform: Plain

Soil profile description

Ap (0-38cm)	Munsell moist (10YR 3/2), dry (5 YR 3/1), clay loam, angular blocky, extremely hard when dry, friable when moist, sticky & plastic when wet, no reaction with HCl, very few faint molting (secondary CC), diffuse smooth boundary
Bti (38-108cm)	Munsell moist (10YR 3/2), dry (7.5 YR 2.5/), clay, angular blocky, extremely hard when dry, friable when moist, very sticky & plastic when wet, dominant & prominent clay coating, slight reaction with HCl, gradual wavy boundary, slickensides.
BC (108-138)	Munsell moist (10YR 4/3), dry (10 YR 3/3), clay, angular blocky, extremely hard when dry, very friable when moist; sticky & plastic when wet, few & distinct clay coatings, clear wavy boundary

Analytical data

Genetic horizon	**Depth**	**pH**	**BD**	**SOC**	**Particle size (%)**			**Texture**
	cm	**H_2O**	**g cm^{-3}**	**%**	**sand**	**silt**	**clay**	**(FAO)**
Ap	0 - 38	7.93	1.47	0.67	31	22	47	C
Bti	39 - 108	7.44	1.43	1.14	18	27	55	C
BC	108-138	7.47	1,36	1.02	45	20	35	SCL
Genetic horizon	**Depth**	**$CaCO_3$**	**Exchangeable base cations**				**CEC**	**BS**
			Ca	Mg	K	Na		
	cm	**%**	**cmol kg^{-1}**					**%**
Ap	0 - 38	0	20.94	4.10	0.45	0.14	46.71	54.88
Bti	39 - 108	8.96	17.00	8.00	0.91	0.46	67.40	39.18
BC	108-138	10.85	13.60	13.20	0.91	0,50	66.48	42.44

Soil type (WRB, 2015): Vertic **Luvisol** (Aric Clayic, Cutanic)

Location: Laelay Maichew, Axum (LMH-2), Profile No. 2

GPS coordinates: N 14° 05' 50", E 38° 46' 91" Land use: Crop agriculture (*Eragrostis tef (Zuccagni) Trotter*)

Altitude: 2070m Slope: 2%

Topography: Gently sloping Landform: Plain

Soil profile description

Ap (0-30cm) Munsell moist (7.5 YR 3/1), dry (5 YR 4/1), sandy clay loam, angular blocky, extremely hard when dry, very friable when moist; very sticky & plastic when wet; no reaction with HCl, clay compaction, diffuse smooth boundary

Bw (30-85cm) Munsell moist (2.5 Y 3/2), dry (10 YR 4/2/), clay angular blocky, extremely hard when dry; friable when moist; very sticky & plastic when wet; common distinct clay coating, clay compaction, no reaction with HCl, clear smooth boundary

Ck (85-130+) Munsell moist (2.5 Y 4/4), dry (2.5 Y 3/3), sandy loam, massive, soft when dry, very friable when moist; slightly sticky & plastic when wet, few fine distinct clay mottles (probably iron & manganese), common distinct $CaCO_3$ coating, , extreme reaction with HCl, clear smooth boundary.

Analytical data

Genetic horizon	**Depth**	**pH**	**BD**	**SOC**	**Particle size (%)**			**Texture**
	cm	**H_2O**	**g cm^{-3}**	**%**	**sand**	**silt**	**clay**	**(FAO)**
Ap	0-30	7.70	1.45	0.46	34	26	40	C
Bw	30-85	7.40	1.40	1.38	46	22	32	SCL
Ck	85-130	7.08	1.15	0.90	26	34	40	C
Genetic horizon	**Depth**	**$CaCO_3$**	**Exchangeable base cations**				**CEC**	**BS**
			Ca	Mg	K	Na		
	cm	**%**	**cmol kg^{-1}**					**%**
Ap	0-30	0	15.48	3.11	0.38	0.04	46.17	41.22
Bw	30-85	9.43	18.00	7.80	0.87	0.43	64.32	42.44
Ck	85-130	8.48	12.00	6.00	0.90	0.51	62.00	31.29

Soil type (WRB, 2015): Dystric Rhodic Vertic **Cambisol** (Aric Clayic, Ochric)

Location: Laelay Maichew, Axum (LMH-10), Profile No. 3

GPS coordinates: N 14° 05' 17.9" E 38° 47' 10.4" Land use: Crop agriculture (*Eragrostis tef (Zuccagni) Trotter*)

Elevation: 2080 m Slope: 2%

Topography: nearly level Landform: Plain

Soil profile description

Ap (0-25cm) Munsell moist (2.5 Y 3/2), dry (7.5 YR 3/1), clay, angular blocky, extremely hard when dry, very friable when moist; very sticky & very plastic when wet, clay compaction, no reaction with HCl, diffuse smooth boundary.

Bi1 (25-51cm) Munsell moist (5 Y 4/2), dry (5 Y 2.5/2), clay, subangular blocky, extremely hard when dry, friable when moist; very sticky & very plastic when wet; clay compaction, no reaction with HCl, diffuse smooth boundary

Bi2 (51-88) Munsell moist (5 Y 3/2), dry (2.5 Y 3/2), silt clay, subangular blocky extremely hard when dry; friable when moist; very sticky & very plastic when wet, clay compaction no reaction with HCl, clear smooth boundary, slickensides

Analytical data

Genetic horizon	Depth	pH	BD	SOC	Particle size (%)			Texture
	cm	H_2O	g cm^{-3}	%	sand	silt	clay	(FAO)
Ap	0-25	7.86	1.11	0.49	42	26	32	C
Bi1	25-51	7.26	1.37	0.87	28	30	42	C
Bi2	51-88	7.24	1.46	0.36	68	28	4	SL1

Genetic horizon	Depth	$CaCO_3$	Exchangeable base cations				CEC	BS
			Ca	Mg	K	Na		
	cm	%	cmol kg^{-1}					%
Ap	0-25	0	18.93	3.85	0.44	0.11	47.25	49.39
Bi1	25-51	8.80	4.02	2.52	0.14	0.16	21.23	32.22
Bi2	51-88	8.90	9.94	0.48	0.14	0.16	19.91	53.83

Soil type (WRB, 2015): Haplic **Vertisols** (Aric, Ochric)

Location: Atsbi Wenberta (ATS-3), Profile No. 4

GPS coordinates N 13° 54' 22.5" E 39°43' 29.2"

Land use: Crop agriculture (*Triticum aestivum L*)

Altitude: 2757 m

Slope: 5%

Topography: nearly level

Soil profile description

Ap (0-18cm)	Munsell moist (7.5 YR 4/4), dry (7.5 YR 3/1), sandy clay, massive, loose when moist; non sticky & non plastic when wet; clay compaction, slight reaction with HCl, diffuse smooth boundary
B1 (18-52cm)	Munsell moist (5 YR 4/4), sandy, subangular blocky very friable when moist; non sticky & non plastic when wet; slight reaction with HCl, diffuse smooth boundary,
B2 (52-80)	Munsell moist (7.5 YR 5/6); silt loam; subangular blocky; very friable when moist; non sticky & non plastic when wet, slight reaction with HCl, diffuse smooth boundary
Bt (80-140^{+})	Munsell moist (7.5 YR 5/6); silt, angular blocky; very friable when moist; non sticky & non plastic when wet, very few fine faint mottles; slight reaction with HCl; diffuse smooth boundary

Analytical data

Genetic horizon	**Depth**	**pH**	**BD**	**SOC**	**Particle size (%)**			**Texture**
	cm	**H_2O**	**g cm^{-3}**	**%**	**sand**	**silt**	**clay**	**(FAO)**
Ap	0-18	7.26	1.18	2.41	43	24	33	CL
B1	18-52	7.45	1.19	0.26	42	19	39	CL
B2	52-80	7.37	1.29	1.85	21	31	48	C
Bt1	80-140	7.47	1.28	0.96	7	26	67	C
Genetic horizon	**Depth**	**$CaCO_3$**	**Exchangeable base cations**				**CEC**	**BS**
			Ca	Mg	K	Na		
	cm	**%**	**cmol kg^{-1}**					**%**
Ap	0-18	0	16.08	5.31	0.52	0.30	41.21	54.20
B1	18-52	8.60	4.32	5.68	0.05	0.43	62.20	16.84
B2	52-80	9.20	2.48	3.96	0.17	0.15	11.14	60.64
Bt1	80-140	9.20	8.24	5.62	0.18	0.14	24.17	58.66

Soil type (WRB, 2015): Luvic Ferrtic **Nitisols** (Aric, Ferric)