CZECH UNIVERSITY OF LIFE SCIENCES PRAGUE

Faculty of Tropical AgriSciences

Towards sustainable land-use management: impacts of agroforestry on soil biodiversity and ecosystem services

BACHELOR'S THESIS

Prague 2024

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Declaration

I hereby declare that I have done this thesis entitled Towards sustainable land-use management: impacts of agroforestry on soil biodiversity and ecosystem services independently, all texts in this thesis are original, and all the sources have been quoted and acknowledged by means of complete references and according to Citation rules of the FTA.

In Prague 18.4.2024

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Anna Šilhánková

Acknowledgements

I would like to express my gratitude to the supervisor of my bachelor thesis Ing. Nikola Teutscherová, Ph. D. for her guidance, patience and willingness to help in my time of need during the creation of my work. I would like to acknowledge and thank Ing. Marie Kalousová for her help and guidance in the laboratory and consultations.

Abstract

Biodiversity in agricultural landscapes, plays a critical role in sustaining agroecosystems by increasing resilience and promoting ecosystem services. Soil biota, although often overlooked but essential, significantly contribute to soil fertility (Kutílek & Nielsen 2015). Their diversity and abundance are strongly influenced by land-use management and crop selection. Recent studies suggests that agroforestry (AF) holds significant promise as a sustainable land-use management approach, with potential benefits for soil health and productivity. This thesis aims to examine how AF practices influence soil biota diversity, with a specific focus on microbial communities of fungi and bacteria. Utilizing next-generation sequencing, this study compares the diversity of bacterial and fungal communities based on Shannon's entropy and Pielou's evenness index across varying land-use systems, including alley cropping AF practice, conventional field (CF) and tree nursery (TN). Specifically, the observed locations were TN, CF and seven distinct locations in the AF, each varying in distance from the tree rows. Six samples were collected for each location. Furthermore, the examination extends to determine how microbial diversity responds to increasing distance from AF tree rows. The findings indicate that bacterial diversity does not significantly differ among the studied locations, while fungal biodiversity increases when the AF system is compared to the TN and CF. However, further study is needed to fully understand the impact of AF on soil biota, including the examination of species composition and time-dependent changes in soil biota.

Keywords: alley cropping, microbial diversity, next-generation sequencing, soil bacteria, soil fungi, sustainable agriculture

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List of the abbreviations used in the thesis

C - Carbon

N -Nitrogen

SDG - Sustainable Development Goal

AF - Agroforestry

ES - Ecosystem Services

DNA - Deoxyribonucleic acid

SOC - Soil organic carbon

TN - Tree nursery

CF - Conventional field

CBD - Convention on Biological Diversity

FAO -Food and Agriculture Organization

PAR – Platform for Agrobiodiversity Research

WWF – World Wildlife Fund

1. Introduction

Global land-use management faces interconnected challenges threatening environmental sustainability and human well-being. Population growth, urbanization, and increasing demand for food, fuel, and fibre have led to widespread land degradation, deforestation, and biodiversity loss. Conventional agricultural practices, dominated by monocultures and reliant on artificial inputs, are widely recognized as the core of food production (Šimek et al. 2019a). But at the same time, recent research recognizes these practices as unsustainable, leading to nutrient depletion, loss of soil organic matter, deteriorating soil health, and escalating dependence on synthetic inputs. The consequences of such unsustainable practices extend beyond mere agricultural concerns, encompassing broader environmental degradation and socio-economic instability.

Addressing these challenges requires a transition towards more sustainable land-use practices, that mitigate soil erosion, water pollution, and enhance soil biodiversity, crucial for long-term soil fertility and ecosystem health (Wooliver et al. 2022). Soil organisms play a vital role in soil formation, functioning, greenhouse gas regulation and carbon sequestration (Plaster 2014; Wooliver et al. 2022). Their presence and diversity in soil serve as indicators of soil health. They also enhance plant nutrient uptake and promote plant health through nutrient cycling processes and diverse symbiotic relationships. Understanding soil ecosystem interactions is essential for effective land-use strategies promoting soil biodiversity and ecosystem services resulting in more resilient and sustainable food production.

Agroforestry (AF) is a promising sustainable alternative to intensive conventional agriculture. By integrating trees and shrubs into agricultural landscapes, agroforestry simultaneously provides ecosystem services and ecological benefits and supports livelihood and food security in both rural and urban settings (Kohli et al. 2007; Jose 2009; Sridhar & Bagyaraj 2017; Beule et al. 2022).

The aim of this thesis is to assess the positive impact of agroforestry on mitigation biodiversity loss, which is generally associated with agricultural land.

2. Literature Review

2.1. Biodiversity

Biological diversity, or biodiversity is a key concept in ecology and has importance on both local and global scales (Haahtela 2019). The Convention on Biological Diversity (CBD) (2011) defines biodiversity as “the variability among living organisms from all sources including, *inter alia,* terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems”. Biodiversity varies greatly with the location, habitat and species being surveyed (Orgiazzi et al. 2016). In general terms, biodiversity tends to be highest at the equator and decreases at higher latitudes (Ricklefs et al. 1995). Therefore, habitats at the equator, such as rain forests, usually have the highest biodiversity and habitats at the north or south poles, such as the polar desert, have the lowest biodiversity.

The biodiversity of current world is the result of billions of years of evolution, influenced by both natural processes and an increasing amount of human activity (CBD 2000). Unfortunately, the present biodiversity is declining. This is not entirely new phenomenon, but the reasons for the current drop in biodiversity are quite different from previous extinction events. This drop in biodiversity has been accelerated by anthropoid activity and while humans are not responsible for all of the recent biodiversity decline, there is strong evidence that humans are affecting the world on a global scale and causing the loss of many important species through species overexploitation, agriculture and land conversion (CBD 2000, 2011; WWF 2018). This loss of biodiversity could have catastrophic consequences for our society as biodiversity is crucial for our health, food security and overall wellbeing (CBD 2000; WWF 2018). Therefore, it is important that we understand how the extinction of species or addition of invasive species impacts the ecosystems we rely on (McCann 2000).

Fortunately, the public sector and government have finally realised that biodiversity conservation is important and are starting to act accordingly. Governments have signed several regional and international agreements to fight environmental destruction and biodiversity loss. There has been the United Nations Conference on the Human Environment in Stockholm in 1972, the United Nations Conference on Environment and Development in Rio de Janeiro in 1992 and more recently, the United Nations Biodiversity Conference in Montreal in 2022. The Convention on Biological Diversity issued in Rio de Janeiro in 1992 was the first global agreement on the preservation and sustainable use of biodiversity (CBD 2000). Now the success the agreements depends on the individual governments and their shared efforts to introduce policies, set rules, and guide public sector to conserve and sustainably use biodiversity. But it all depends on the individual citizens to make the right choices towards a healthy biodiversity (CBD 2000).

Ecosystem biodiversity is crucial for life on this planet (CBD 2011). By increasing the local biodiversity, the environment becomes more resilient and stable (Haahtela 2019), but with a certain level of dynamic stability that protects the ecosystem processes from changes in the environment and / or stress (Mooney et al. 1995). In addition, increasing biodiversity improves ecosystem properties such as carbon sequestration or productivity (Chapin et al. 2001).

2.1.1. Biodiversity in Research

Biodiversity studies encompass a spectrum of dimensions, which are crucial for understanding species distribution and variability at different levels. This variability of all living organisms encompasses diversity at three different levels (i) genetic diversity describing the total genetic information of all species or the genetic variation between species or individuals, (ii) species diversity defining the variety of species, and (iv) ecosystem diversity (Swingland 2013).

The species diversity is further characterized at three levels (i.e. alpha, beta and gamma) (Whittaker 1960). Alpha diversity refers to the total number of species found in a particular area, often in a specific habitat. Gamma diversity represents the richness of species across large areas of land, and beta diversity compares the species diversity between two areas (e.g. different communities, ecosystems, and habitats) (Andermann et al. 2022).

To analyse, understand and capture interesting trends a plethora of methods were invented. Scientists examine biodiversity based on its (i) species abundance, which determines the total number of organisms in the observed area (ii) species richness, which determines the number of different species in the area (iii) species diversity analyses both abundance and diversity to comprehend species distribution in the area, and (iv) species evenness, which determines how equally distributed the species in the area are.

This can be measured by many different biodiversity indices, which help find promising trends and dependencies in an ecosystem or a location (e.g. Shannon's diversity index, Simpson's diversity index, Pielou's evenness index, Chao's index). Pielou's evenness index can be used to examine the evenness of abundance of different species. Shannon's entropy, which describes the uncertainty of picking two same species, is used to understand the richness and evenness of distribution of species abundance And Chao's index can be used for capturing the richness of species in a location.

2.2. Soil Biodiversity

Soils are home to the highest biodiversity of any region on Earth (Kutílek & Nielsen 2015; Orgiazzi et al. 2016). Edaphon, all living organisms in any soil, contains an enormous number of species including nematodes, algae, amoeba, fungi, actinomycetes, and bacteria (Plaster 2014). Only the zooedaphon (the animals living in soil) itself represents up to 23 % of the total number of living organisms (Decaëns et al. 2006). Interestingly, the large numbers of organisms described to live in soils are only a small fraction of the estimated absolute number (Brussaard 1997; Chapin et al. 2001). Figure 1 describes estimated numbers of species known and unknown of the major soil animal taxa. One of the reasons for this knowledge gap is the difficulty of identifying species. Identifying species diversity in soils is challenging due to limited taxonomic expertise and labour-intensive sampling, extraction, and identification of the soil biota (Chapin et al. 2001). The soil biota is then also often overlooked and not given enough recognition for their role in determining soil properties and production potential (Brussaard 1997). All the upper mentioned problems result in a lack of attention paid to soil biota by conservation biologist. Although the previously emphasized need for biodiversity applies perhaps even more to soil biodiversity, since soil biodiversity is crucial to the survival of soil and, by extension, the life on Earth (Kutílek & Nielsen 2015). Yet, higher plants and vertebrates are often favoured over these soil microorganisms (Decaëns et al. 2006; Kutílek & Nielsen 2015; Bottinelli et al. 2015).

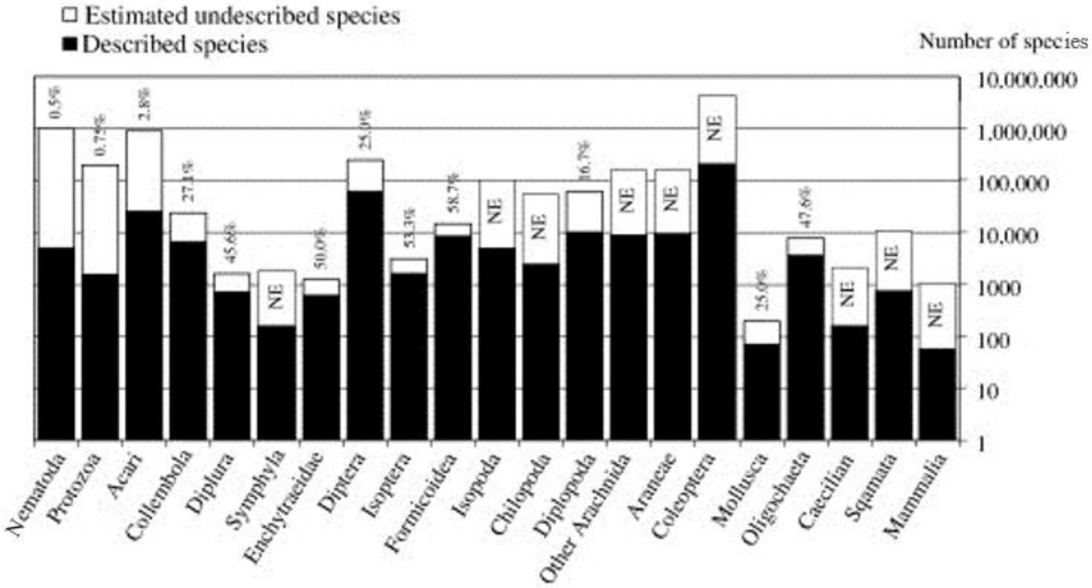

Figure 1: Estimated numbers of described and un-described species for major soil animal taxa (Decaëns et al. 2006)**, NE = no estimation available.**

Soil organisms which are responsible for the development of soils (Kutílek & Nielsen 2015) are generally categorized into the following categories: (i) microorganisms, (ii) microfauna, (iii) mesofauna and (iv) macrofauna (Table 1). They have a significant impact on the hydrology, aeration, and gaseous composition of the soil (Brussaard 1997) and can be affected both at local and regional levels, by change in the environment, soil structure and chemical composition, introduction of invasive species and climate change (Bongers 1990; Ruess 1995; Chapin et al. 2001). Geographic patterns of soil biodiversity are in general poorly explored. Chapin et al. (2001) state that the previously mentioned general idea that species diversity is at its highest at the equator does not apply for all soil organisms. And while some organisms, such as termites and ants, follow this rule (Folgarait 1998), other organism's biogeographic patterns are no so simple. Most soil organisms (e.g. nematodes, earthworms, enchytraeids) have rather irregular global distribution with no clear patterns; additionally, most geographical patterns of soil organisms are fairly unexplored (Lavelle 1983; Brussaard 1997). This lack of familiarity with the global distribution of soil biota hinders accurate predictions for species loss, introductions, soil restoration species, and the impact of global change on soil species (Chapin et al. 2001).

Table 1: Major soil organisms, in order of increasing body width, number of species found in soils and estimated total number of species that exist in all habitats (data from Brussaard 1997; Chapin et al. 2001; Orgiazzi et al. 2016).

Taxonomic group	Body width	Number of described soil species	Estimated number of species in all habitats
Microorganisms			
Bacteria	1 - 2 µm	15, 000	1,000,000
Fungi	3 - 100 µm	18,000 - 35,000	1,500,000
Microfauna			
Protozoa	15 - 100 µm	1,500	200,000
Nematoda (roundworms)	5 - 120 µm	5,000	400,000 - 10,000,000
Rotifera	24 - 130 µm	250	No estimate
Mesofauna			
Acari (mites)	80 µm - 2 mm	20,000 - 30,000	900,000
Cellembola (springtails)	150 µm - 2 mm	6,500	24,000
Enchytraeidae (pot worms)	500 µm - 4 mm	600	1,200
Macrofauna			
Isoptera (termites)	500 µm - 4 mm	1,600	3,000
Formicoidea (ants)	500 µm - 4 mm	8,800	15,000
Oligochaete (earthworms)	1 - 50 mm	3,627	No estimate
Chilopoda (centipedes)	1 - 50 mm	2,500	No estimate
Diplopoda (millipedes)	1 - 50 mm	10,000	60,000

In the soil horizon, soil organisms primarily inhabit the top 60 cm of soil (see Figure 2 for bacteria). Their global composition and quantity are shaped by: (i) vegetation, (ii) physical and chemical attributes of soil, (iii) local climate conditions, and (iv) the interplay among soil organisms (Chapin et al. 2001; Orgiazzi et al. 2016). Subterranean life notably concentrates near plant roots, known as the rhizosphere, where they can find enough organic matter to thrive (Lavelle 1997; Plaster 2014). Various studies consistently demonstrate a decline in both abundance and diversity as one moves away from plants (Chapin et al. 2001). This highlights the significant influence of plant-root interactions on the presence and variety of soil life. Based on their size, soil organisms live in one or more soil pores, e.g. the empty spaces in the soil free of organic matter, which can be either aerobic or anaerobic (Kutílek & Nielsen 2015). Generally, microorganisms (e.g. bacteria and fungi) cover soil aggregates and particles, microfauna (e.g. protozoa and roundworms) live in water-filled pores, mesofauna (e.g. mites and pot worms) live in air-filled pores, and macrofauna (e.g. ants and centipedes) live in the surface litter or in nests and burrows (Lavelle et al. 2006).

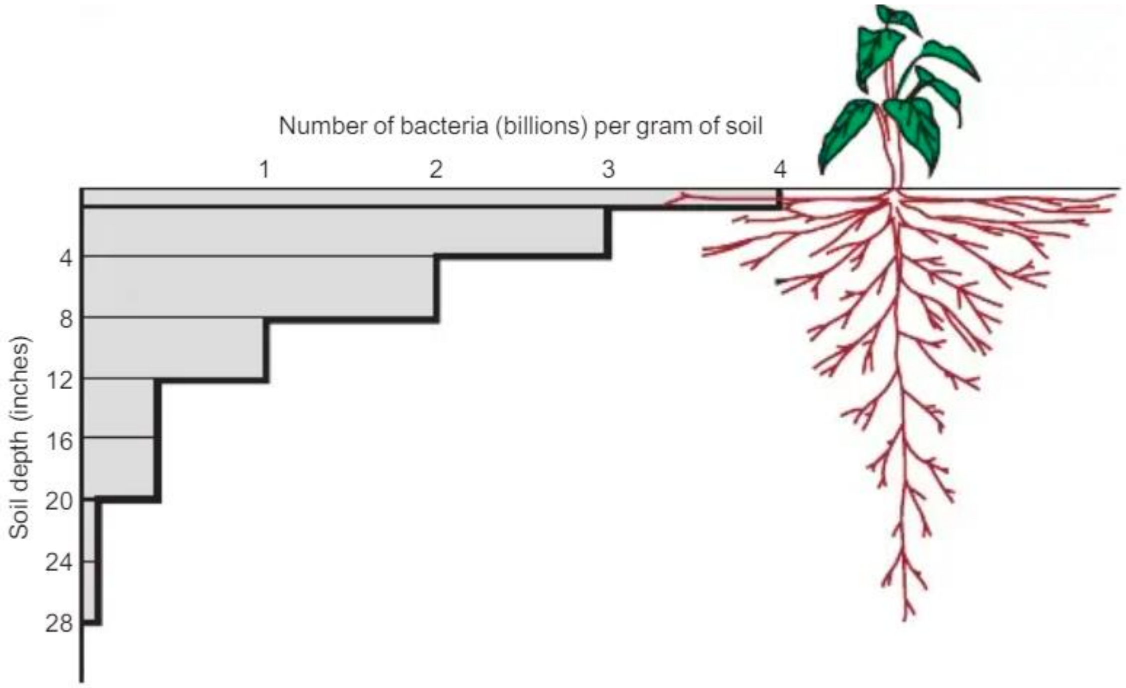

Figure 2: Soil bacteria distribution in soil horizon (Plaster 2014)**.**

2.2.1. Bacteria

Soil bacteria form a dynamic and diverse community with a significant role in soil health. Their average body width is between 1 and 2 µm (Chapin et al. 2001), making them the second smallest soil creatures after viruses (i.e. bacteriophages) in soil. They are unicellular prokaryotic organisms that are predominantly present in three shapes: (i) rod (bacilli), (ii) sphere (cocci), and (iii) spiral (spirilla) (Hoorman 2016a). Many bacteria are pleomorphic, meaning that they can take on multiple forms depending on the attachment of new cells. They can form into pairs, chains, and clusters (Orgiazzi et al. 2016). Additionally, mobile bacteria develop a whip-like structure (flagellum).

Bacteria are the most abundant and variable soil organisms (Plaster 2014), representing organisms that thrive in aerobic or anaerobic conditions and many heterotrophic and autotrophic species. They primarily occur in soils as a thin biological film on the surface of solid particles and near plant roots (Hoorman 2011; Kutílek &

Nielsen 2015). They are predominant in tilled and generally more disturbed soils with rapid nutrient cycling, low carbon to nitrogen (C : N) levels and near annual plants (Hoorman 2011, 2016b; Šimek et al. 2019b).

Due to their small size, bacteria quickly develop and adapt (Hoorman 2011). Because of this, the size of their communities is very much tied to the surrounding environment and changes depending on soil physical and chemical properties (e.g. moisture, temperature, organic matter content) as well as soil management type, and thrive when food, water and ideal conditions are available. Some bacteria species are very fragile and may die by slight changes in the soil environment. Others are extremely resilient and can withstand severe heat, cold or drought (Orgiazzi et al. 2016). Some bacteria depend on specific plant species, forming a symbiotic relationship with them (e.g. species of the *Frankia* genus) (Lavelle & Spain 2003; Šarapatka 2014).

The general classification of soil bacteria examines several phenotypic traits of the organisms, e.g. their cell morphology (rods, spheres, spirals, and others), cell wall structure (gram negative × gram positive), their ability to move and the presence of endospores (Lavelle & Spain 2003; Hoorman 2011). Other classifications are based on the species physiology (autotrophic × heterotrophic) and their function (e.g. aerobic or anaerobic, cellulolytic, nitrifiers, N-fixers).

Bacteria act as decomposers, mutualists with plants (e.g. nitrogen-fixing *Rhizobia*), pathogens (e.g. *Agrobacterium, Erwinia, Pseudomonas*), and lithotrophs or chemoautotrophs that obtain energy from compounds like nitrogen and sulphur (Hoorman 2011; Šarapatka 2014). They are critical in the biogeochemical cycles of plants, and their interactions in the rhizosphere determine plant health and soil fertility (Hayat et al. 2010). Plant growth promoting rhizobacteria (PGPR) play a significant role by synthesizing compounds for plants, facilitating nutrient uptake, preventing diseases, soil improvement and nutrient solubilization (Hayat et al. 2010).

2.2.2. Fungi

Fungi, eukaryotic organisms ten times larger than soil bacteria, are primarily present in the soil in the form of mycelium consisting of multicellular fungi linked into a long chain of hyphae (Lavelle & Spain 2003; Kutílek & Nielsen 2015; Orgiazzi et al.

2016). Unicellular fungi (e.g. *Saccharomyces*), widely called yeasts, are present in the soil only in small quantities (Lavelle & Spain 2003).

Fungi are generally dominant in forest and peat soil, preferring soils with low pH values, preferably soils in undisturbed areas with slow nutrient recycling time and high C : N ratio (Lavelle & Spain 2003; Plaster 2014; Kutílek & Nielsen 2015; Hoorman 2016b). They are most abundant in topsoil down to a depth of 10 cm; with greater depth, their numbers decrease (Šarapatka 2014).

The dominant function of soil fungi is to serve as heterotrophic decomposers, but they also act as mutualists, pathogens, and predators (e.g. fungi feeding on nematodes) (Lavelle & Spain 2003). As heterotrophs, fungi energy and carbon sources come from the chemical breakdown of bonds in organic substances. Fungi are known for their capability to decompose lignin and cellulose, organic materials forming rather complex and strong bonds, making them hard to disintegrate. However, the ability of fungi to penetrate the material’s surface and to work from within enables faster decomposition of these organic materials (Plaster 2014). Additionally, fungi produce hormones, vitamins, enzymes, or certain antibiotics that benefit plant growth and health and reduce plant root diseases (Kutílek & Nielsen 2015; Hoorman 2016b).

Another feature of soil fungi is their capability to form a symbiotic relationship with plants, known as mycorrhiza. Mycorrhizae fungi serve as mediators in the nutrient (e.g. P, Zn, Cu) and water intake of plants; they expand the range and permeability of the plant’s root system and protect against plant pathogens (Plaster 2014; Šarapatka 2014; Kutílek & Nielsen 2015). In exchange for these services, mycorrhizae fungi have access to glucose and sugar produced by plants (Šarapatka 2014; Kutílek & Nielsen 2015; Orgiazzi et al. 2016).

2.2.3. Algae and Cyanobacteria

Other microorganisms living in the soil are algae and cyanobacteria. These autotrophic organisms are primary producers in soil (e.g. plants, lichens, moss, photosynthetic bacteria, and algae). They produce organic compounds necessary for their function by photosynthesis. Due to this capability to transform solar radiation into energy, essential for their lives, most algae, and cyanobacteria dwell at or near the soil surface (Kutílek & Nielsen 2015; Meena et al. 2019).

Soil algae are mostly unicellular eukaryotic organisms most abundant in moist, mineral rich soils (Šarapatka 2014). They are understood as the primary soil pioneers who stand behind the weathering of rocks and sediments and the establishment of a soil's primary layer, promoting the life of the following heterotrophic organisms (Rahmonov et al. 2015). Another beneficial feature of algae is the aeration and oxygenation of soil environments (Šarapatka 2014; Kutílek & Nielsen 2015).

Cyanobacteria, also known as blue green algae, even though they are unrelated to any of the algae species, are prokaryotic organisms found in both unicellular and multicellular forms (Závodská 2006; Orgiazzi et al. 2016). They are the first organisms known to produce oxygen, playing a crucial role in the past Earth's oxygenation (Orgiazzi et al. 2016). In addition to their ability to aerate and oxygenate the soil, some cyanobacteria are also capable of nitrogen fixation (Šarapatka 2014; Orgiazzi et al. 2016).

2.2.4. Microfauna

Kutílek and Nielsen (2015) describe soil microfauna as the most prevalent zooedaphon group, consisting of protozoa, rotifers and, to a lesser degree, nematodes. In general, the width of their bodies does not exceed 0.1 mm, and their length varies from a few micrometres (e.g. protozoa) to one millimetre (e.g. nematodes) (Orgiazzi et al. 2016). They are heterotrophic organisms, playing a crucial role in nutrient cycling as decomposers, predators and somehow less as pathogens. Their primary function in the soil is to break down organic matter into less complex compounds, thereby supporting nutrient cycling. Other roles of soil microfauna, associated mainly with nematodes, include mixing soil particles and forming pathways in the soil (Plaster 2014). These organisms are dependent on water and can be found primarily in water films around soil particles and in water-filled pores. However, their specific distribution in soil horizon varies organism by organism. Numbers of protozoa, a diverse group of unicellular eukaryotic organisms of different shapes and features, are particularly high near plant roots, where they prey on rhizobacteria. Nematode populations, consisting of multicellular microscopic non-segmented organisms with cylindrical body tapering at both ends, occur predominantly in the upper layer of the soil profile, but there are cases of bacterivorous nematodes being localized more than 3.6 km below the Earth’s surface (Plaster 2014; Orgiazzi et al. 2016). According to Orgiazzi et al. (2016), soil nematodes divide into five main feeding groups: nematodes consuming bacteria, fungi, omnivores, parasites, and predators, and they can be beneficial or unwanted organisms in the soil. The last organism of microfauna mentioned by Kutílek & Nielsen (2015) are rotifers, a multicellular organism with a body divided into three parts: head, trunk, and foot, living predominantly near the soil surface. They are bacterivorous and algivorous organisms feeding by filtering water or browsing, promoting the nutrient cycling in soil by consuming others or being consumed (Orgiazzi et al. 2016).

2.2.5. Mesofauna

Mesofauna consist of mites (Acari), springtails (Collembola), pot worms (Enchytraeidae), as well as, to much lesser extent, Diplura, Pseudoscorpionida, and Protura species, their body sizes range in between 0.1 to 2 mm in body width, and 0.1 to approximately 10 mm in body length (Lavelle & Spain 2003; Orgiazzi et al. 2016). The most significant of them are microarthropods (e.g. springtails and mites), invertebrates with aerial systems of respiration, meaning that they live in aerobic conditions in water-free pores near the soil surface or in the surface litter. They function primarily as decomposers and soil engineers, mixing soil and affecting soil porosity (Plaster 2014). Acari, with jointed legs and strong external skeletons, a distinctive feature characteristic to all arthropods, are classified into three major groups: (i) microphytophages eating bacteria, algae and fungi, (ii) macrophytophages eating leaf litter, and (iii) predators preying on small invertebrates (Lavelle & Spain 2003; Šarapatka 2014). They are abundant in forest soils, where the upper layer of the soil is less disturbed; on the contrary, their populations decrease in tilled and more disturbed soils of arable land (Plaster 2014; Šarapatka 2014). Collembola, inhabiting all continents and capable of living in extreme conditions, are the phylogenetically oldest known hexapods (Šimek et al. 2019b). Their characteristic features are a ventral tube (*tubus ventralis*) and a jumping apparatus (*furcula*) on their abdomen; however, this feature is sometimes reduced or missing in species living deeper in the soil (Šarapatka 2014; Šimek et al.

2019b). Apart from being known as decomposers and soil engineers of arctic and alpine soil, they also regulate mycorrhizal fungi and plant pathogens living in the soil (Šimek et al. 2019b). The last significant family, the Enchytraeidae, resembling small earthworms, with whom they relate, also play a significant role in soil health (Šimek et al. 2019b). They also share with earthworms similar roles in the soil, participating in decomposition, nutrient cycling, and formation of soil microstructure, and they also have an impact on soil-borne pathogens (Pelosi & Römbke 2018).

2.2.6. Macrofauna

Soil macrofauna consists of species most known for their role as soil engineers. Their burrowing and nesting mix the soil, affecting its structural dynamics and enhancing soil drainage and oxidation properties (Folgarait 1998; Plaster 2014; Kutílek & Nielsen 2015; Bottinelli et al. 2015). They are a diverse group of invertebrates, ranging in body width from 2 to 20 mm (Orgiazzi et al. 2016). The most important of them are, according to Bottinelli et al. (Bottinelli et al. 2015), earthworms, termites, insects, and ants. Other macrofaunal species mentioned by Šarapatka (2014) and Orgiazzi et al. (2016) are arachnids (e.g. spiders), myriapods (e.g. millipedes, centipedes), and gastropods (e.g. snails and slugs). Apart from their primary function as soil engineers, they also regulate microbial communities in soil, and thereby protecting plants from parasitic and herbivorous species and accelerating the plant succession rate (Brussaard 1997; Bottinelli et al. 2015). Most macrofauna live in the surface litter and vegetation or the top layer of soil. Other species are part-time soil inhabitants, using soil as a place for their nests and shelters (Decaëns et al. 2006; Šarapatka 2014; Orgiazzi et al. 2016).

2.3. Interactions Among Soil Organisms

Soil organisms influences in its unique way its surroundings (see Table 2), but also form relationships with each other, determining the soil nutrient cycling, decomposition, and ecosystem dynamics (Šarapatka 2014; Šimek et al. 2019c). These various food, symbiotic, parasitic, or competitive relationships then form the entire underground ecosystem, which, if in balance, positively affects the quality and health of the soil and can be used as indicators of soil quality (Lavelle et al. 2006; Plaster 2014; Šimek et al. 2021). One possible way to display energy and carbon flow between soil organisms is by the utilization of the soil food web (for a simplified version, see Figure 3), also known in its more straightforward form as a soil food chain. The energy flow is initiated by the primary producers (e.g. plants and algae), who, by photosynthesis, convert atmospheric carbon into organic carbon and turn solar energy into chemical energy, storing it in the form of sugars and other different energy-rich compounds (Plaster 2014). The energy and organic carbon, then, by consumption, flows from the primary producers to primary consumers (i.e. organisms consuming plants), who are then being eaten by secondary consumers (i.e. predators) and so forth, moving the energy and C up in the chain or web (Plaster 2014; Šarapatka 2014). Another influential group present is then decomposers (i.e. saprophytes), who break down the organic matter of dead organisms or animal wastes into nutrients, ensuring the carbon cycle.

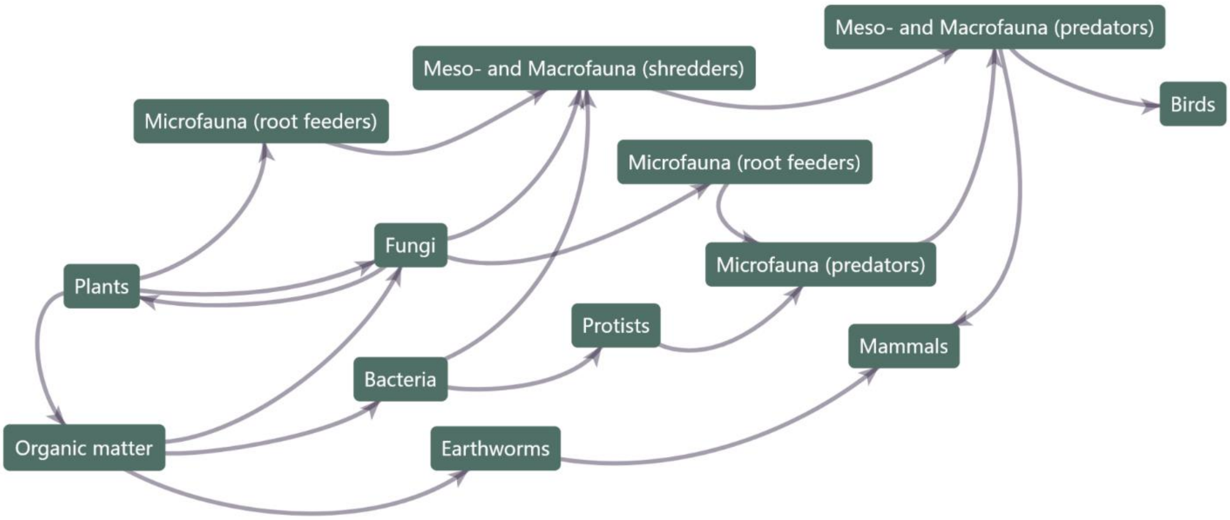

Figure 3: Simple food web representing some of the possible feeding connections in soil (data from Orgiazzi et al. 2016)**.**

Table 2: Major soil organisms, their classification, and their functions in soil (data from Chapin et al. 2001; Šarapatka 2014; Šimek et al. 2019a).

Classification	Taxonomic group	Main Function in Soil
Microorganisms	Bacteria	Decomposition of organic matter, mutualists, nutrient cycling, pathogens, pathogen suppression, soil structure improvement
	Fungi	Decomposition of organic matter, mycorrhizal symbiosis, pathogens, pathogen suppression, soil aggregation
	Algae and Cyanobacteria	Carbon sequestration, nitrogen fixation, photosynthesis, soil pioneers, soil stabilization
Microfauna	Protozoa	Nutrient cycling, predation on microorganisms
	Rotifers	Nutrient cycling, predation on microorganisms, soil aeration
	Nematoda (roundworms)	Increase N availability, plant and animal parasitism, predation on microorganisms
Mesofauna	Acari (mites)	Enhancing microbial growth, fragmentation of organic matter, phytophages, predation on microorganism, soil structure improvement
	Cellembola (springtails)	Decomposition of organic matter, nutrient cycling, predation, soil structure improvement
	Enchytraeidae (pot worms)	Decomposition of organic matter, enhancing microbial growth, nutrient cycling, soil aeration
Macrofauna	Isoptera (termites)	Decomposition of organic matter, enhancing microbial growth, nutrient cycling, soil aeration, soil engineers
	Formicoidea (ants)	Decomposition of organic matter, enhancing microbial growth, nutrient cycling, soil aeration, soil engineers
	Oligochaete (earthworms)	Decomposition of organic matter, nutrient cycling, soil aeration, soil engineers, soil structure improvement
	Chilopoda (centipede)	Decomposition of organic matter, predation on other soil organisms (e.g., insects, earthworms), soil aeration, soil stabilization
	Diplopoda (millipedes)	Decomposition of organic matter, nutrient cycling, soil aeration, soil stabilization

2.4. The Role of Edaphon in Ecosystem Services

As was already mentioned, edaphon provides many essential functions (see Table 2), many of which play a role in the sustainable functioning and stability of soil ecosystem, which is beneficial for its efficiency and productivity (FAO & PAR 2011; Orgiazzi et al. 2016; Kaushal et al. 2017). These functions are then a part of necessary ecosystem services (ES) provided by the soil fauna and flora (see Table 3), but it is required to add that the ecosystem services are not mutually exclusive and often overlap. The ecosystem services are an indirect economic value of soil organisms (the long-term impact of their activity that is not directly harvested) (Decaëns et al. 2006). And according to Orgiazzi et al. (2016), they are defined as: “Tangible and intangible benefits that humans obtain from ecosystems”. They are more difficult to evaluate but can be economically beneficial, and if they are not present, their substitutes can become expensive (Decaëns et al. 2006). The specific ecosystem functions and services of edaphon are then greatly dependent on the type of land management practices and the type of vegetation cultivated, as they influence the soil state and determine if the soil conditions will be appropriate for the survival of many soil species (Brussaard 2012). Generally speaking, the more an agroecosystem conserves biodiversity, the higher the number of ES it delivers (Varah et al. 2013).

Table 3: Ecosystem services and functions provided by soil biodiversity (data from Chapin et al. 2001; Lavelle et al. 2006; Orgiazzi et al. 2016)**.**

Ecosystem service type	Ecosystem functions
Provisioning	Contribution to all plant production for food, fuel, and fibre Infiltration and storage of water in soil pore system
Regulating	Biological population regulation Bioremediation of wastes and pollutants Carbon cycle regulation Control of potential pests and diseases Mitigation of global change Modification of the hydrological cycle (e.g. mitigation of floods and droughts, erosion control) Regulation of atmospheric trace gases Translocation of nutrients, particles, and gases
Supporting	Absorption and storage of water Generation of soil structure and fertility Nutrient cycling (e.g. decomposition, humidification, regulation of nutrient losses) Pedogenesis Production of hormones and enzymes Renewal of soil structure and fertility Retention and delivery of nutrients to plants Stimulation of symbiotic activity in the soil

2.5. Soil Biodiversity in Agricultural Landscapes

The conflict between agricultural production and biodiversity conservation is pervasive. Human land use, including agriculture, shapes most of the Earth’s land surface, impacting organisms living in these areas. This land conversion has recently moderated, and the area of agricultural land has stabilized. However, to address the needs of Earth’s growing population, it was necessary to increase the agricultural production of current agricultural land. The intensification of agricultural land poses a danger and negatively affects species richness (Benton et al. 2003; Decaëns et al. 2006; Beckmann et al. 2019). The market system in agriculture and the insufficient care and protection of the soil legally result in poor agricultural practices, which degrade soils and deplete them of organic matter, which is necessary for soil organisms (Kutílek & Nielsen 2015; Šimek et al. 2021). For these soils to be profitable, farmers often rely on artificial fertilizers, pesticides, fungicides, irrigation, and heavy machinery, which further damage the soil's natural ability to regenerate, making the soil unsuitable for many organisms (Ricklefs et al. 1995; Benton et al. 2003; Šimek et al. 2021). Chapin et al. (2001) add that even if agricultural management is based on less intensive practises (e.g. no-till, minimal-chemical input), it shows some level of disturbance to the soil and its edaphon. Fortunately, the lower the level of disturbance in an agricultural system is, the more it supports species richness (i.e. the total number of organisms/ individuals in the area) (Donald 2004). Ultimately, the species richness may be influenced by the land-use intensification differently across taxa, production type, land management history, and climate conditions (Beckmann et al. 2019). In each case, it is necessary to enhance biodiversity conservation on agricultural land as it, as described by Thrupp (2000), is in many areas of human life and agricultural production beneficial (see Figure 4). Fortunately, many environmental regulations of developed countries today see the need to conserve soil biodiversity and currently fight back against the use of external inputs (e.g. fertilizers, pesticides), to restore the natural functioning of the soil biota, which may solve issues connected with soil health, salinity and degradation (Brussaard 2012; Pandey et al. 2019).

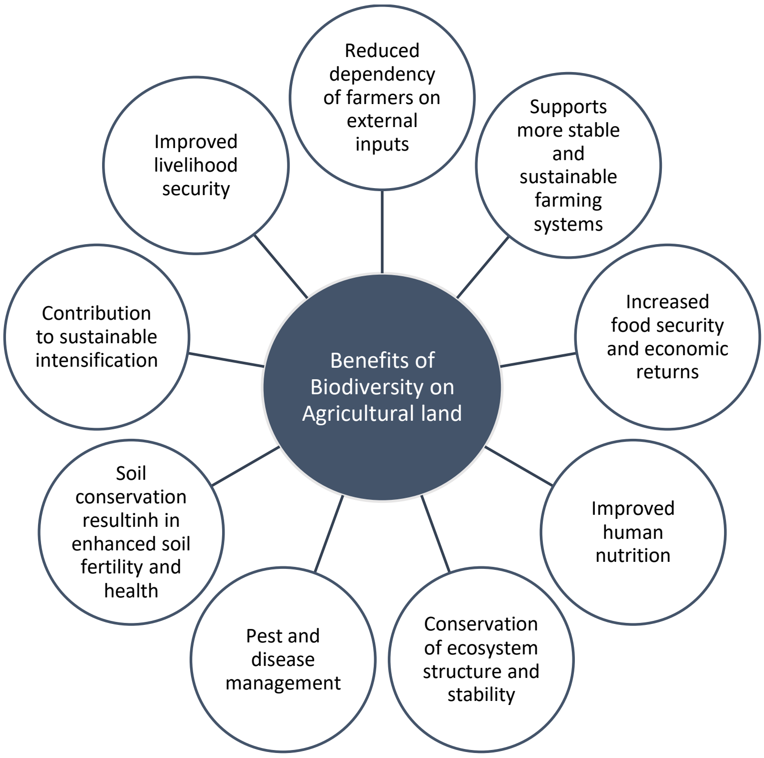

Figure 4: Benefits of biodiversity on agricultural land (data from Thrupp 2000, Kaushal et al. 2017).

2.5.1. Impact of Monocultures and Chemical Inputs on Soil Biodiversity

Large-scale and intensive farming practices heavily rely on synthetic inputs such as pesticides and fertilizers, favour maximizing crop yields for economic gain, often prioritizing monocultures for streamlined management and increased production efficiency. This intention to profit comes at the cost of biodiversity loss in these systems. Conventional agricultural land then quickly becomes barren ground with poor soil structure, leading to soil water and wind erosion, ultimately killing the soil and its biota (Kutílek & Nielsen 2015). To fight this, farmers add more and more chemicals into the soil, leading to further unsustainable land-use practices.

The need to use pesticides in intensive agriculture stems from their effectiveness in reducing, destroying, or mitigating pests, primarily based on their toxicity. However, this toxicity also results in adverse effects of these chemicals on humans, livestock, wildlife, insects, and soil organisms (Orgiazzi et al. 2016; Šimek et al. 2019a, 2021). These effects occur through direct contact or consumption or indirectly through water, soil, air, and food contamination. Individual soil organisms exhibit varying responses to chemicals depending on their properties, the properties of given chemicals and the surrounding soil (Orgiazzi et al. 2016; Šimek et al. 2019a). Roundworms and rotifers typically absorb chemicals through their body surface and food, whereas organisms with exoskeletons of chitin (e.g. insects) primarily ingest pollutants from their diet in the soil. Organic and inorganic fertilizers, often applied in conventional agriculture to help keep up crop production, can positively or negatively impact the number of soil organisms (Orgiazzi et al. 2016). To limit their negative impact on soil, their use in agriculture must be limited and based on the current state of nutrients in the soil, as an extensive excess of any nutrient becomes toxic for the plant and other organisms (Šimek et al. 2019a).

Finally, the soil biota in intensive agriculture is affected by the cultivation of monocultures, defined as an agricultural technique based on the repeated production of the same crop on the same plot of land (Orgiazzi et al. 2016; Šimek et al. 2021). Monoculture cultivation or rotation of only a few crops with a high market value will result in a short-term profit. However, it eventually depletes the soil, does not promote soil aggregation, and increases the demand for mineral fertilizers and pesticides

(Gordon et al. 2018; Šimek et al. 2021). Insufficient or no crop rotation further supports the growth of weeds, pests, and pathogens in the land and relate to low soil biodiversity (Altieri & Nicholls 2008; Orgiazzi et al. 2016; Sheppard et al. 2020; Šimek et al. 2021).

2.6. Agroforestry and Sustainable Agriculture

Sustainable agricultural systems require technology and practices that are environmentally friendly, accessible to farmers, and increase food yield (Pretty 2008). It promotes practices such as crop rotation, organic farming, conservation tillage, crop diversification, and integrated pest management to enhance soil health, reduce water usage, and protect biodiversity. According to some authors, these environmentally friendly food production practices will replace conventional agricultural practices, as they may be the solution for sustainable food security that does not deplete natural resources (Glick 2018). Additionally, sustainable agriculture aligns with several United Nations Sustainable Development Goals, including zero hunger (SDG 2), clean water and sanitation (SDG 6), responsible consumption and production (SDG 12), climate action (SDG 13), and life on land (SDG 15), making it one of the focus targets in the agricultural sector. Examples of sustainable agriculture include (i) organic farming, where farmers prioritize natural methods over synthetic inputs; (ii) community-supported agriculture, which connects consumers directly with farmers; (iii) permaculture, which mimics natural ecosystems; (iv) aquaponics, which integrates fish farming with hydroponic plant cultivation, and (v) agroforestry (AF).

AF is a form of sustainable intensification of the land that grows more food whilst minimizing its environmental impact (Godfray et al. 2010; Beckmann et al. 2019). Šimek et al. (2019b) define AF as: “A form of landscape utilization in which the traditional cultivation of agricultural crops or animal grazing combines with the simultaneous cultivation of woody plants in the same area”. It represents a sustainable land management strategy that can generate income, promotes food security, biodiversity protection, and ecosystem services (e.g. nutrient cycling, reduced soil erosion), and mitigate climate change (Varah et al. 2013; Orgiazzi et al. 2016; Mosquera-Losada et al. 2018; Sheppard et al. 2020). Using and growing trees on agricultural land also addresses and helps mitigate climate change and improves the system’s resilience (van Opstal et al. 2021). However, to achieve AF full potential, farmers need to design AF systems according to the area and manage these systems in a way that minimizes negative interactions (e.g. competition for light, space, nutrients, and water) between the trees, crops and livestock (Cannell et al. 1996; Thevathasan & Gordon 2004; Kohli et al. 2007; McAdam et al. 2008; Varah et al. 2013; Lojka et al. 2022). The main AF types, categorized according to their components, are silvoarable AF and silvopastoral AF (Šimek et al. 2019a). Other commonly used types of AF systems are described in Table 4. AF, along with the integration of trees into agricultural landscapes, is widely accepted as beneficial by numerous studies, with its overall influence on temperate and tropical climates generally regarded as positive (see Table 5). Šimek et al. (2019b) describe the positive impact of AF on soil structure, ultimately reducing soil erosion, utilization of nutrients in soil, biodiversity, carbon sequestration, and soil remediation. Additionally, AF positively influences microclimate (e.g. temperature) and water scarcity in its area and helps with the mitigation of extensive floods, limiting the water erosion of soil (Jose et al. 2004; Orgiazzi et al. 2016; Dupraz et al. 2018).

Table 4: Descriptions of agroforestry systems and practices (data from Dupraz et al. 2018; Šimek et al. 2019b; Lojka et al.

Agroforestry system	Agroforestry practice	Tree location	Description
Silvoarable AF	Alley cropping	Trees inside parcels	Intercropping of annual crops in between rows of trees or shrubs planted in a field, providing multiple yields from the land.
	Forest farming	Trees inside parcels	Cultivating shade tolerant plants under a forest canopy to optimise productivity.
Silvopastoral AF	Wood pastures	Trees inside parcels	Grazing of livestock on agricultural land intercropped with trees to improve animals' welfare.
	Forest grazing	Trees inside parcels	Grazing of livestock within forested area to control undergrowth and improve forest health.
Agrosilvopastoral AF	Alternating cropping and grazing	Trees inside parcels	Integration of trees, crops, and livestock on the same piece of land to optimize resource use and productivity.
Permanent crop AF - silvoarable	Orchard intercropping	Trees inside parcels	Cultivating crops inside an orchard system to maximize land use efficiency and biodiversity.
Permanent crop AF - silvopastoral	Orchard grazing	Trees inside parcels	Controlled grazing of livestock within orchards, utilizing animals for weed and pest control while enhancing soil fertility.
Field boundary AF	Windbreaks and shelterbelts	Trees between parcels	Tree lines at the edges of land /around field protecting crop or soil against wind erosion
	Hedgerows	Trees between parcels	Linear plantings of shrubs, trees, or a combination, typically serving as barriers, wildlife habitats, and soil stabilizers.
	Riparian buffer strips	Trees between parcels	Woody stands along waterways or water bodies adjacent to areas of crops, aimed at mitigating pollution, erosion, and providing wildlife habitat.
Urban AF	Home gardens and allotments	Trees in settlements	Small-scale AF integrated into residential areas, providing food, shade, and cultural significance.

Table 5: The main functions of AF with the type of effect and their area of impact (data from Kohli et al. 2007; McAdam et al. 2008).

The main type of area of impact	Effect (positive / negative)	Description of function	Example of goods and services
Production	Positive	Creation of biomass	Trees: timber, fruits, nuts, fodder, firewood, cork Crops: fruits, vegetables, grain, seeds, biofuel, fodder Animals: meat
Habitat	Positive	Provision of habitat for conservation and maintenance biological diversity	Habitat diversity Species diversity Shelter diversity Mechanical support
	Negative	Habitat disruption	Invasive behaviour of some of the introduced species
Regulation	Positive	Maintenance of essential ecological processes and life support systems	Soil and water conservation Reduced nutrient leaching Reduced fire risk Carbon sequestration Weed and pest management Improvement of microclimate Phytoremediation
	Negative	Resource competition	Competition for nutrients, water, light, and space
		Chemical interference	Allelopathy
Cultural	Positive	Opportunities for reflection, cognitive development, and recreation	Cultural heritage Landscape enhancement Recreation
		Alleviating poverty and enhancing food security	Job creation Improved production

2.6.1. The Challenges of the Implementation of Agroforestry in Temperate Zone

The current agricultural sector in Europe has been afflicted by concerns such as overproduction, declining farmer earnings, rural depopulation, and environmental degradation caused by intensive production practices (Palma et al. 2007a). Amidst these challenges, AF emerges as a promising alternative, offering multifunctional land use that can mitigate environmental impacts while supporting livelihoods.

However, despite demonstrating its effectiveness, ecological benefits, and extensive use of AF in tropical regions, AF encounters significant impediments in its implementation, particularly in temperate regions such as Europe and the USA (Mosquera-Losada et al. 2018). Administrative institutions within national governments frequently promote separate land-use management (i.e. forestry, agriculture, and husbandry), dismissing AF as an unconventional practice (McAdam et al. 2008). As a result, there are few supportive policies to promote AF practices, enhancing the challenges created by the historical intensification of farming systems in the 20th century, during which many AF practices (e.g. hedgerows, forest grazing) disappeared from the temperate regions such as Europe and the USA (Quinkenstein et al. 2009; Mosquera-Losada et al. 2018). Moreover, the compartmentalization of land-use management into agriculture, forestry, and animal husbandry has overlooked the potential synergies and advantages that AF offers in terms of enhancing ES and promoting sustainable land use. This fragmented approach not only hinders the integration of AF practices but also perpetuates a narrow perspective on land management that fails to harness the full potential of AF.

Furthermore, quantifying the global extent of AF systems presents its own set of challenges. Current estimates suggest that approximately 1.6 billion hectares worldwide are under AF, with the majority (78 %) located in tropical regions (Nair et al. 2021). However, accurately delineating these areas remains problematic due to the lack of standardized procedures for identifying irregular stands of trees intermixed with crops.

2.6.2. Agroforestry and Ecosystem Services

AF, as a form of land management, can affect the community structure and activity of all soil organisms and, therefore, their contribution to the ecosystem functions and services of the soil (see Table 3) (Orgiazzi et al. 2016). Additionally, as outlined in Table 5, the tree’s inclusion alongside other components of AF contributes to their ecosystem functions and adds cultural, regulatory and production values to the land (Lojka et al. 2022). AF generally provides more ES than conventional agriculture without trees does while at the same time not limiting or reducing the lands’ productivity (Santiago-Freijanes et al. 2018; Lojka et al. 2022). However, it is necessary to add that not every implementation of AF systems is beneficial, and the knowledge of the land-use history is needed, to decide whether the AF system implementation exhibits loss or gain of ES. For example, the establishment of AF by thinning primary and natural forests (e.g. tropical forest) is associated with the loss of some ES and on the other side, AF established on previously open land (e.g. meadow, arable land) gains new ES (Martin et al. 2020).

AF systems have been hypothesised to provide a variety of ES, but until relatively recently, the scientific data to back up these anticipated advantages was lacking (Jose 2009). Some of the essential ES which mentioned in Chapter 2.6 as benefits of agroforestry are: (i) biodiversity conservation, (ii) soil conservation, (iii) enhancement of air and water quality, (iv) carbon capture and storage, and (v) cultural services (Jose 2009; Skok 2023). The role of AF in biodiversity conservation is be discussed in the following Chapter (2.6.3). The rest will be briefly described here.

2.6.2.1. Soil Conservation

This ES of agroforestry is especially needed to reach some of the SDGs (e.g. SDG 2, 13) and ensure food security for future generations. As agroforestry systems become more diverse structurally and functionally, they become more effective in soil conservation, reducing erosion and surface runoff (Torralba et al. 2016). The introduction of AF practices leads to long-term improvements in soil quality, positively impacting future yields and sustainability (Jose 2009). Studies have consistently shown that AF enhances soil health by improving physical and chemical properties (Thevathasan & Gordon 2004; Kohli et al. 2007; Torralba et al. 2016). This improvement is attributed to agroforestry’s ability to increase soil organic matter and the tree root systems’ capacity to enhance soil C content, mitigate compaction, and improve porosity, infiltration, and water retention (Šimek et al. 2019a). Such contributions are particularly vital in sloping, drought-prone areas where soil erosion is significant (Torralba et al. 2016).

2.6.2.2. Enhancement of Air and Water Quality

AF practices may serve used as a mitigation strategy against various environmental pollutants, including water contamination, odour pollution from intensive livestock operations, noise pollution, and air particle pollution (Jose 2009). By addressing issues such as fertilizer runoff and leaching into underground water sources, AF plays a crucial role in safeguarding water quality (Jose 2009). Additionally, AF enhances water usage reduction and water retention capacity of the soil, which results in AF being a much more water-sustainable agroecosystem than conventional agriculture (Kohli et al. 2007). Furthermore, AF promotion of microclimate through factors like temperature moderation, humidity regulation, and shade provision by perennial crops add to the water usage effectiveness (Skok 2023). In terms of air quality, AF practices contribute to particulate matter filtration, enhance oxygen production, and air pollution buffering, thereby improving overall air quality (Jose 2009). Tree lines then on agricultural land are also connected to decrease in evaporation, and their orientation based on the dominant wind direction can reduce wind speed, sheltering the annual crops from physical damages caused by strong winds (Quinkenstein et al. 2009).

2.6.2.3. Carbon Capture and Storage

Since 1850, a considerable amount of C stored in soil has been released, due to the depletion of soil organic carbon (SOC) in agricultural land, into the atmosphere (Cardinael et al. 2017). This pressing problem of declining C reserves in terrestrial ecosystems and the resulting increase in greenhouse gas levels has prompted global attention (Kohli et al. 2007). Carbon sequestration (CS) from the atmosphere in soil and vegetation provided by the trees’ and shrubs’ presence in AF systems addresses this problem and can increase the SOC stock in soil (Jose 2009; Cardinael et al. 2017). Research indicates that AF systems have a substantial capacity for CS, surpassing other agroecosystems devoid of trees (FAO & PAR 2011; Varah et al. 2013). However, the extent of CS under AF systems can vary. Factors such as the type of AF (see **Error! Reference source not found.**), species composition, date of establishment, age of component plants, geographic location, prevailing environmental conditions and land-use history influence CS rates (Jose 2009; Feliciano et al. 2018). Generally, AF systems demonstrate superior CS compared to monocultures of agricultural crops, except for AF plantations compromising fast-growing trees, where the impact may be neutral (Šimek et al. 2019a).

2.6.2.4. Cultural Services

The presence of trees within agroecosystems enhances landscape diversity and contributes to their aesthetic appeal, offering opportunities for recreation and supporting the cultural value of these areas (Skok 2023). This is particularly evident in AF systems characterized by high biodiversity and cultural significance, defined by Moreno et al. (2018) as high nature and cultural value AF, which have the potential to attract tourism and provide recreational spaces. Studies examining farmers' perspectives on AF establishment in Europe emphasize the significant role of trees' aesthetic value in driving AF adoption and tree implementation (Lojka et al. 2022; Skok 2023).

2.6.3. Impact of Agroforestry on Soil Biodiversity

The positive impact and ecosystem service of AF on biodiversity conservation is based on its ability to provide diverse habitats and improve the microclimate and soil properties. AF leverages the unique characteristics of trees, such as their size, longevity, and diverse structure (e.g. variety of tissues), to create habitats for a wide array of organisms, enhancing ecosystem complexity and environmental heterogeneity (Burgess 1999; Dupraz et al. 2018; Mosquera-Losada et al. 2018). The subsequent interaction of trees with other components of agroforestry (i.e. crops, animals, crops and animals) then only enhances this effect of trees further (Palma et al. 2007b; McAdam et al. 2008). In agricultural land, the tree rows of AF, which are not mechanically disturbed, create migration corridors, barriers, shelters and feeding areas, which support a variety of species, such as earthworms, spiders, mammals, amphibians, invertebrates, mosses, and plants (Dupraz et al. 2018).

The implementation of AF in comparison with agricultural land enhances the soil properties, trees provide shade and intercept rainfall, and fallen leaves and branches cover the soil, altogether creating a favourable condition for edaphon to thrive in their area of impact (Rigueiro-Rodríguez et al. 2009; Pauli et al. 2010; Orgiazzi et al. 2016). Furthermore, regular pruning, followed by mulching during dry seasons, adds to and maintains the soil’s organic matter composition, promoting higher soil moisture content necessary for microbial activity and survival (Orgiazzi et al. 2016). In addition to the enhancement of organic matter entering the ecosystem, the plant diversity promoted by AF improves the stability and supply of resources produced by plants, which also benefits the soil communities (Orgiazzi et al. 2016). Recent AF research underscores the intricate interplay between tree arrangement and soil biological activity, with some tree species exhibiting a stronger influence than others and reinforcing the concept of trees as hotspots, where trees emerge as dynamic focal points of edaphon activity (Rigueiro-Rodríguez et al. 2009; Orgiazzi et al. 2016). For example, mature dehesa, a kind of agrosilvopastoral AF where trees, animals and herbaceous cover are combined, is believed to be the most diverse environment created by humans in Europe, providing a shelter for a wide range of invertebrates, birds, and other wildlife and flora (McAdam et al. 2008; Moreno & Pulido 2009; Rigueiro-Rodríguez et al. 2009). However, it is crucial to note that the abundance of edaphon differs considerably between the area near AF trees and the rest of the AF land (Banerjee et al. 2016). For instance, fungi and bacteria numbers in the upper layer of the soil rises with decreasing distance from trees and the same rule applies to earthworms (Thevathasan & Gordon 2004; Pauli et al. 2010; Šimek et al. 2019a). However, the research on microbial biomass in AF systems yields contradictory and inconclusive findings; some authors highlight the positive effects of AF on microbial biomass, while others argue that the effects are negative (Banerjee et al. 2016). Furthermore, the impact of AF can vary across taxa examined, and at the same time, it depends on which ecosystem the AF effect on biodiversity is compared to (Torralba et al. 2016).

3. Aims of the Thesis

The bachelor’s thesis aim was to find out how the adoption of alley cropping AF system influences the soil microbial diversity. The specific objectives were:

- To evaluate the diversity of soil microbial communities of bacteria and fungi among the alley cropping AF system, conventional agriculture, and tree nursery;
- To assess the heterogeneity of microbial diversity within the alley cropping AF system.

To achieve this goal, I set up the following hypotheses:

1. The alley cropping AF system is expected to have higher soil microbial diversity in comparison with tree nursery (TN) and conventional field (CF).
2. The presence of trees will have stronger impact on soil fungal communities than on soil bacteria.
3. The microbial diversity will decrease with the increasing distance from the tree line within the alley cropping system.

4. Materials and Methods

4.1. Site Description

The sample collection was conducted at the Michovka experimental station agroforestry system of the Silva Tarouca Research Institute for Landscape and Ornamental Gardening (VUKOZ), located in the Central Bohemian Region of the Czech Republic, specifically in Průhonice at coordinates 49.9919669N, 14.5784056E. The experimental station was established in 1992, has 23 ha of land and serves as an area for horticultural and bioenergy research. The AF utilizing alley cropping practice is a new addition to this experimental site, it was implemented between 2018 and 2019 and covers 0.6 ha in dimensions of 60 x 100 m of the Michovka's land.

This silvoarable system emerged through the transformation of an ageing alley tree nursery, initially established in 2004-2005. At the time of establishing the silvoarable system, the trees had reached the age of 15-16 years. Presently, the remaining trees stand at 20 years old. The implementation involved the removal of excess trees using chain saws, with trunks transported by horses and stumps homogenized through robust rotary cutting. The resultant layout features alley cropping AF practice with tree lines spaced at intervals of 7, 10, and 15 meters, utilizing diverse tree species including maple (Acer spp. and *Acer campestre*), linden (*Tilia* spp.), ash (*Fraxinus* spp., *Fraxinus excelsior*), rowan (*Sorbus torminalis*), Turkish hazel (*Corylus colurna*), and others (see Appendix 1).

The experimental site encompasses not only the newly established silvoarable system but for experimental reasons also incorporates part of the preserved over-aged tree nursery (see Appendix 2). Additionally, in collaboration with neighbouring agro-cooperative (AGRO Jesenice u Prahy a.s.), researchers from VUKOZ can use their arable parcel managed as conventional agriculture with intensive crop rotation (CF), located 50 meters away from the AF system, a contrasting land use system for comparison. For research purposes both the arable soil in between the tree lines of AF and neighbouring conventional agricultural field are managed in the same way, using the same crop, type of management and inputs. This meticulously designed site offers a unique opportunity to explore the intricate dynamics between agroforestry practices and conventional agriculture, shedding light on sustainable land management strategies' potential benefits and challenges.

4.2. Sample Collection and Processing

4.2.1. Sample Collection

The soil sample collection from the described site (see Chapter 4.1) was conducted in May 9, 2023. A total of 54 samples were collected from three contrasting land use systems, including AF, TN and CF. The samples were systematically numbered from 1 to 48, with additional samples labelled as 4b, 10b, 16b, 22b, 28b, and 34b. The allocation of samples was as follows:

- Samples numbered 1-36, along with the control samples, were extracted from the established alley cropping system (AF).
- Samples numbered 37-42 were obtained from the over-aged tree nursery (TN).
- Samples numbered 43-48 were taken from the neighbouring field of conventional agriculture (CF).

The first 42 soil samples from AF were collected in 3 line transects (see Figure 5), which intersect the established tree lines. The selected AF area was established with two different widths of the alleys, the first where the spacing of the trees in the alley cropping system was 15 meters apart (AF15) and the second where the spacing of the trees in the alley cropping system was 10 meters apart (AF10). From each, samples were collected on both sides of the tree row accordingly to the line transects. In the AF15, 6 samples were collected at the distance of 7.5 m (AF15-7.5), 5 m (AF15-5), 2.5 m (AF15-2.5), and 0 m (AF15-0) from the tree line. The same number of samples (n = 6) was also collected in AF10 at each of the following distances: 5 m (AF10-5), 2.5 m (AF10-2.5) and 0 m (AF10-0) from the tree line. Then 6 samples were taken from the tree nursery (TN) and another 6 samples were collected from the conventional field (CF) (see Figure 6). In the TN and CF, sampling points were collected randomly at least 40 m apart.

The collection of soil samples was conducted using a hand-held auger probe. We collected the top 15 cm of soil into clear resealable plastic bags. Following, the extraction the samples were promptly transferred to a freezer to preserve their biological content until further DNA extraction.

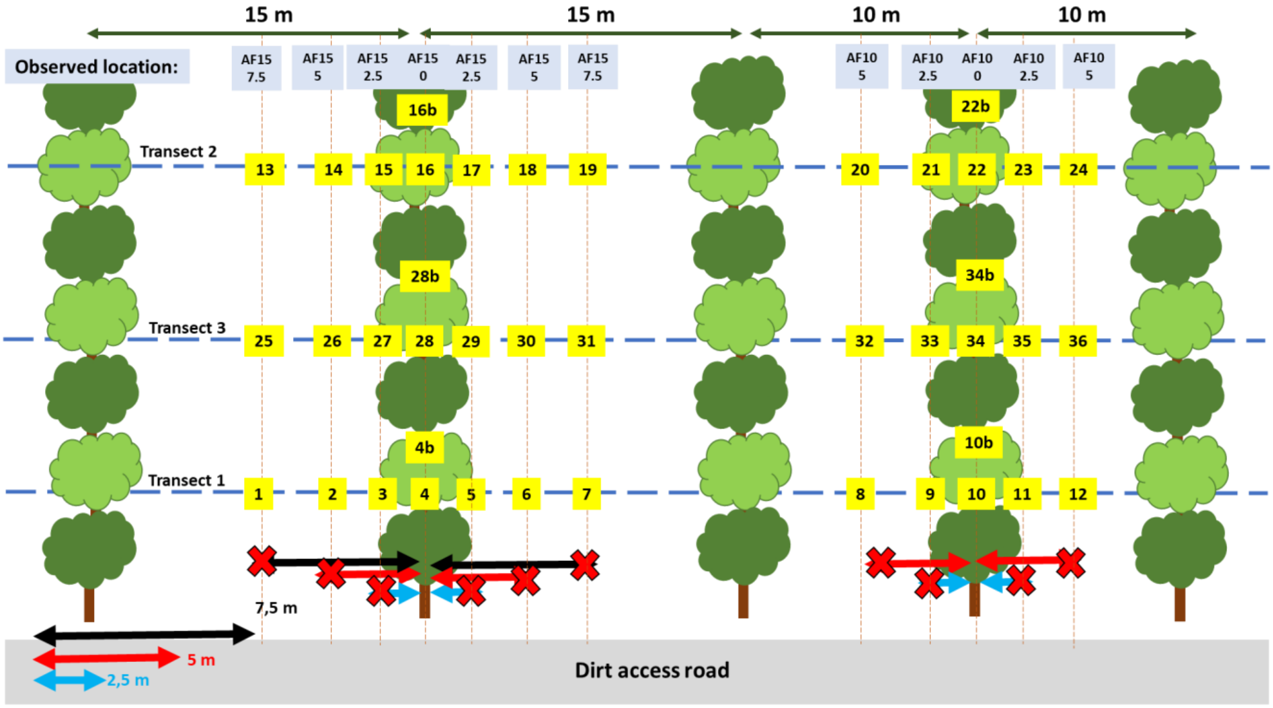

Figure 5: Sampling design of AF at Michovka experimental station, VUKOZ.

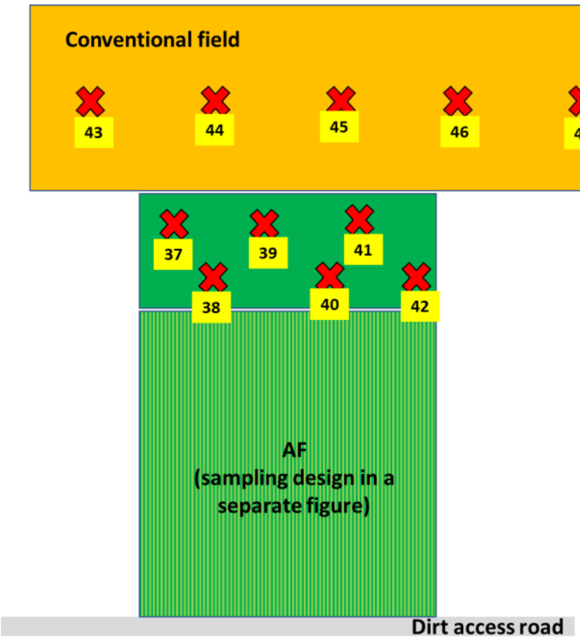

Figure 6: Sampling design of TN at Michovka experimental station, VUKOZ and neighbouring CF.

4.2.2. DNA Extraction

The DNA extraction was conducted at the Laboratory of Molecular Genetics at the Faculty of Tropical AgriScience at the Czech University of Life Sciences Prague. To extract the microbial genomic DNA from the collected soil samples the DNeasy PowerSoil Pro Kit (Qiagen, Germany) was used. This extraction kit allowed for efficient lysis of bacteria and fungi from the samples, enabling the isolation of high yields of bacterial and fungal DNA from the samples. All the steps in the protocol for the DNeasy PowerSoil Pro Kit designed by Qiagen were precisely followed (see Appendix 3).

The evaluation of the ongoing quality of the DNA extraction concentration was determined by the device Nanodrop One Spectrophotometer (Thermo Fisher Scientific, USA). For this purpose, 2 µl of the extracted material was used. First, the blank sample of 2 µl of elution buffer was measured, followed by the individual extracted DNA samples. Final quality control of all extracted DNA samples was performed on fluorometer Qubit 4 (Thermo Fisher Scientific, USA).

The extracted microbial DNA was then sent to SeqMe laboratory for metagenomic next generation sequencing analysis in the volume of 50 µl with DNA concentration of 3 ng/ µl. To maximize the visibility of targeted taxa of interest the amplification of the fungal internal transcribed spacer (ITS2_short) and bacterial 16S rDNA gene of V4 region (16S_V4) primers described in Table 6 were used.

Table 6: Selected primers for amplification

	Primer	References
Bacteria	515f	(Caporaso et al. 2011)
	806r	(Caporaso et al. 2011)
Fungi	ITS4r	(White et al. 1990)
	gITS7f	(Ihrmark et al. 2012)

4.3. Data Analysis

Obtained results of bacterial and mycorrhizal diversity indices (Shannon's entropy and Pielou's evenness index) from each soil sample, from the metagenome analysis by SeqMe were processed in IBM SPSS Statistics version 29.0.2.0. First, all data were checked for homogeneity by the tests of homogeneity of variances and normality by the Shapiro-Wilk test of normality ($p \leq 0.05$). As the conditions of these two tests were not fulfilled by majority of the data, a nonparametric statistical method (Kruskal-Wallis test) was chosen to examine the data. The Kruskal-Wallis test tested the following null hypothesis:

The distribution of Shannon's entropy and Pielou's evenness is the same across categories of location (AF15-7.5, AF15-5, AF15-2.5, AF15-0, AF10-5, AF10-2.5, AF10-0, TN, CL).

The tested fields for statistics were Shannon's entropy and Pielou's evenness index values. The selected grouping variable was the location (see **Error! Reference source not found.**). Then the data were evaluated by the Tukey Post Hoc test. The significance values have all been adjusted by the Bonferroni correction for multiple tests. Differences were considered significant at $p \leq 0.05$.

The data analysis firstly compared whether there are a statistically different values of Shannon's entropy and Pielou's evenness among the two most contrasting AF locations (closest to the tree line: AF15-0 and AF10-0, and farthest from the tree line: AF15-7.5 and AF10-5) and the two conventional land uses (TN and CF). Then comparison of the individual locations in AF was performed, to determine how the locations differ from each other and whether there is a significant difference ($p \leq 0.05$) between them.

5. Results

5.1. Comparison of AF with Conventional Field and Tree Nursery

5.1.1. Bacteria Diversity

No significant difference in Shannon’s entropy values for soil bacteria were detected among the tested locations (see Table 7). However, a significantly different values ($p \leq 0.05$) in between the observed types of land management were observed for the Pielou’s evenness index, where the bacterial evenness was significantly higher ($p \leq 0.05$) in the conventional field (CF) than the in AF locations, which were closest from the tree line (AF15-0 and AF10-0) (see Table 7). The high Pielou’s evenness index of the CF was also significantly different ($p \leq 0.05$) from the sampling distance in AF 5 m away from the tree line in the AF system, where the tree lines were 10 m apart (see Table 7).

Table 7: Shannon’s entropy and Pielou’s evenness index for bacteria in two contrasting locations of AF system (the middle of cultivated alley and within the tree line) and two contrasting conventional agricultural systems (TN and CF). Mean values followed by standard errors (n = 6); different lower-case letters indicate significant difference ($p < 0.05$) between the different land management systems.

Observed locations[1]	Shannon’s entropy			Pielou’s evenness		
AF15-7.5	8.522	0.190	a	0.930	0.002	ab
AF15-0	8.4723	0.135	a	0.924	0.003	b
AF10-5	8.544	0.175	a	0.923	0.003	b
AF10-0	8.399	0.135	a	0.922	0.005	b
TN	8.697	0.144	a	0.930	0.005	ab
CF	8.596	0.157	a	0.935	0.003	a

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[1] Observed location: AF15-7.5, agroforestry location, where tree rows were 15 m apart, samples taken 7.5 m away from tree rows; AF15-0, agroforestry location, where tree rows were 15 m apart, samples taken 0 m away from tree rows; AF10-5, agroforestry location, where tree rows were 10 m apart, samples taken 5 m away from tree rows; AF10-0, agroforestry location, where tree rows were 10 m apart, samples taken 0 m away from tree rows; TN, tree nursery; CF, conventional field.

5.1.2. Fungi Diversity

The comparison of Shannon's entropy values for AF (AF15-7.5, AF15-0, AF10-5 and AF10-0) with NT and CF showed a significantly higher ($p \leq 0.05$) value in location AF10-5, when compared to the two conventional land uses (NT and CF) (see Table 8). In case of Pielou's evenness index significantly higher ($p \leq 0.05$) values were found in AF locations, which were farthest from the tree line (AF15-7.5 and AF10-5), when compared to the NT (see Table 8).

Table 8: Shannon's entropy and Pielou's evenness index for fungi in two contrasting locations of AF system (the middle of cultivated alley and within the tree line) and two contrasting conventional agricultural systems (TN and CF). Mean values followed by standard errors (n = 6); different lower-case letters indicate significant difference ($p < 0.05$) between the different land management systems.

Observed location[2]	Shannon's entropy			Pielou's evenness		
AF15-7.5	6.760	0.240	ab	0.818	0.009	a
AF15-0	6.414	0.403	ab	0.789	0.026	ab
AF10-5	7.118	0.228	a	0.836	0.014	a
AF10-0	6.675	0.490	ab	0.794	0.033	ab
TN	6.087	0.421	b	0.770	0.019	b
CF	6.372	0.208	b	0.800	0.011	ab

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[2] Observed location: AF15-7.5, agroforestry location, where tree rows were 15 m apart, samples taken 7.5 m away from tree rows; AF15-0, agroforestry location, where tree rows were 15 m apart, samples taken 0 m away from tree rows; AF10-5, agroforestry location, where tree rows were 10 m apart, samples taken 5 m away from tree rows; AF10-0, agroforestry location, where tree rows were 10 m apart, samples taken 0 m away from tree rows; TN, tree nursery; CF, conventional field.

5.2. Effect of Distance from the Tree Rows in Alley Cropping AF

5.2.1. Bacteria Diversity

Within the AF system, effect of the distance from the tree was detected neither in Shannon's entropy, nor in Pielou's evenness index (see Table 9).

Table 9: Shannon's entropy and Pielou's evenness index for bacteria, locations of AF system of different distances from tree rows. Mean values followed by standard errors (n = 6); different lower-case letters indicate significant difference ($p < 0.05$) between the different land management systems.

Observed location[3]	Shannon's entropy			Pielou's evenness		
AF15-7.5	8.527	0.190	a	0.930	0.002	a
AF15-5	8.439	0.140	a	0.924	0.006	a
AF15-2.5	8.472	0.109	a	0.928	0.005	a
AF15-0	8.473	0.135	a	0.924	0.003	a
AF10-5	8.544	0.175	a	0.923	0.003	a
AF10-2.5	8.431	0.109	a	0.924	0.003	a
AF10-0	8.399	0.135	a	0.922	0.005	a

5.2.2. Fungi Diversity

Significantly different ($p \leq 0.05$) values of Pielou's evenness were observed between the different types of AF locations (see Table 10). Where the Pielou's evenness is significantly higher ($p \leq 0.05$) for sampling distance 5 m away from the tree line in the AF location where the tree lines were 10 m apart, than for the sampling distance 0 m away from the tree line of AF location, where the tree lines were 15 m apart. No significant difference in Shannon's entropy values for soil fungi were detected among

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[3] Observed location: AF15-7.5, agroforestry location, where tree rows were 15 m apart, samples taken 7.5 m away from tree rows; AF15-5, agroforestry location, where tree rows were 15 m apart, samples taken 5 m away from tree rows; AF15-2.5, agroforestry location, where tree rows were 15 m apart, samples taken 2.5 m away from tree rows; AF15-0, agroforestry location, where tree rows were 15 m apart, samples taken 0 m away from tree rows; AF10-5, agroforestry location, where tree rows were 10 m apart, samples taken from 5 m away from tree rows; AF10-2.5, agroforestry location, where tree rows were 10 m apart, samples taken from 2.5 m away from tree rows; AF10-0, agroforestry location, where tree rows were 10 m apart, samples taken from 0 m away from tree rows.

the tested locations (see Table 10). However, the location in AF10 5 m away from the trees shows slightly higher value for the observed index than the nearest location to the tree line in AF15 (AF15-0). Although the differences among locations in Shannon's entropy were not significant, the location within the highest Pielou's evenness index (AF10-5) corresponds with the highest Shannon's entropy value.

Table 10: Shannon's entropy and Pielou's evenness index for fungi, locations of AF system of different distances from tree rows. Mean values followed by standard errors (n = 6); different lower-case letters indicate significant difference ($p < 0.05$) between the different land management systems.

Observed location[4]	Shannon's entropy			Pielou's evenness		
AF15-7.5	6.760	0.240	a	0.818	0.009	ab
AF15-5	6.571	0.427	a	0.799	0.039	ab
AF15-2.5	6.610	0.292	a	0.800	0.011	ab
AF15-0	6.414	0.403	a	0.789	0.026	b
AF10-5	7.118	0.228	a	0.836	0.014	a
AF10-2.5	6.667	0.267	a	0.813	0.025	ab
AF10-0	6.675	0.490	a	0.794	0.033	ab

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[4] Observed location: AF15-7.5, agroforestry location, where tree rows were 15 m apart, samples taken 7.5 m away from tree rows; AF15-5, agroforestry location, where tree rows were 15 m apart, samples taken 5 m away from tree rows; AF15-2.5, agroforestry location, where tree rows were 15 m apart, samples taken 2.5 m away from tree rows; AF15-0, agroforestry location, where tree rows were 15 m apart, samples taken 0 m away from tree rows; AF10-5, agroforestry location, where tree rows were 10 m apart, samples taken from 5 m away from tree rows; AF10-2.5, agroforestry location, where tree rows were 10 m apart, samples taken from 2.5 m away from tree rows; AF10-0, agroforestry location, where tree rows were 10 m apart, samples taken from 0 m away from tree rows.

6. Discussion

6.1. Microbial Diversity in Various Land-Use Managements

Despite the general positive impacts of AF on soil described in the literature (Jose et al. 2004; Orgiazzi et al. 2016; Dupraz et al. 2018; Šimek et al. 2019a), this study showed limited evidence of AF influence on soil bacterial biodiversity opposing the first set hypothesis. The bacterial community diversity did not differ between the compared AF locations and the two conventional systems (TN and CF), which disagrees with results from a study examining soil bacterial communities in Canadian AF done by Banerjee et al. (2016). This lack of effect of AF in this study could be explained by the universal ability of bacteria to adapt, proposed by Hoorman (2011) and may suggest that, however, the diversity of the organisms did not vary different species composition could evolve across the locations.

On the other hand, a comparison of these systems revealed a positive effect of AF on soil fungi diversity, which is consistent with the first and second hypotheses, highlighting the positive influence of crop diversification (especially the implementation of perennial crops) on the taxa, described by Orgiazzi et al. (2016) and Wooliver et al. (2022). This was further confirmed by the fact that higher values for AF were also recorded for the species evenness of fungi. The documented influence of AF on microbial communities aligns with the prevailing hypothesis that fungal populations potentially derive greater advantages from AF implementations compared to bacteria (Beule et al. 2022). However, interestingly, the diversity and evenness of fungi did not increase in the TN. This could be caused by several factors including the small size, age, and location of the observed area (TN), which might have been influenced by the adjacent CF, consequently impacting the soil properties of TN.

6.2. Microbial Diversity within Alley Cropping AF System

The positive influence of trees on microclimate suggested and proven in research (Jose et al. 2004; Orgiazzi et al. 2016; Dupraz et al. 2018), suggest that the tree rows or individual trees in AF systems should have an impact on the biodiversity and within the tree proximity. However, contradictory to the findings and the third set hypothesis. This study showed that the soil microbial diversity does not increase with the proximity to the three lines. This lack of effect on the bacterial richness might be caused by the small number of samples or by the fact that the individual AF systems are neighbouring each other and the tree rows are quite close. So, it is possible, that the influence of trees had a larger impact and overlapped creating a uniform microclimate condition across the whole alley cropping AF system. Another possible explanation is that the species composition at the individual locations might have shifted and altered based on the changing environment (Banerjee et al. 2016). It would be interesting to see how the situation evolves over time and how different measurements throughout the year report the change in the microbiome. An additional comparison between optimal and extreme conditions could give us a glimpse into whether the ES of AF can create a more resilient system, as the literature suggests (Swamy & Tewari 2017; van Opstal et al. 2021).

Interestingly, not even the anticipated increased diversity of soil fungi near the tree lines was detected. However, this might be due to the fact, that the diversity of fungi was, in general, high at the AF when compared to TN and CF. Or, at the same time, this observed effect could be influenced by the fact, that the alley cropping AF system was not implemented on arable land but was established on land previously covered by tree nursery by felling. This type of AF introduction is generally unexplored and might have a different impact on the soil microbial organisms as we can generally assume that the implementation of ploughed and arable lines in between the tree rows will deteriorate the soil quality of the previously undisturbed area.

7. Conclusion

The hypotheses set were partially proven by this study. The first hypothesis, suggesting that the observed locations in the AF system will have a higher biodiversity than tree TN and conventional field, was partially supported by the results. While the diversity of soil fungi indeed increased in AF locations, the expected higher diversity in soil bacteria was not observed. The second hypothesis was in line with our findings and the third was refuted. This revealed a further need for an additional examination of microbial and specifically biodiversity of bacterial communities with a supplementary study assessing the species present in the microbial community as it might serve for the observation of the different factors shaping the microbiota in soil. Moving forward, several recommendations for future research emerge from our findings. Increased sample size and repetitive sampling would enhance the reliability and generalizability of results. Comparison with older research at the same locations could provide interesting data, showing how the introduction of the alley cropping AF system at the previously wooded lot affects soil and its biota. This may help to create suggestions on how to establish AF system that effectively balances agricultural productivity and environmental goals.

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List of Appendices

Appendix 1

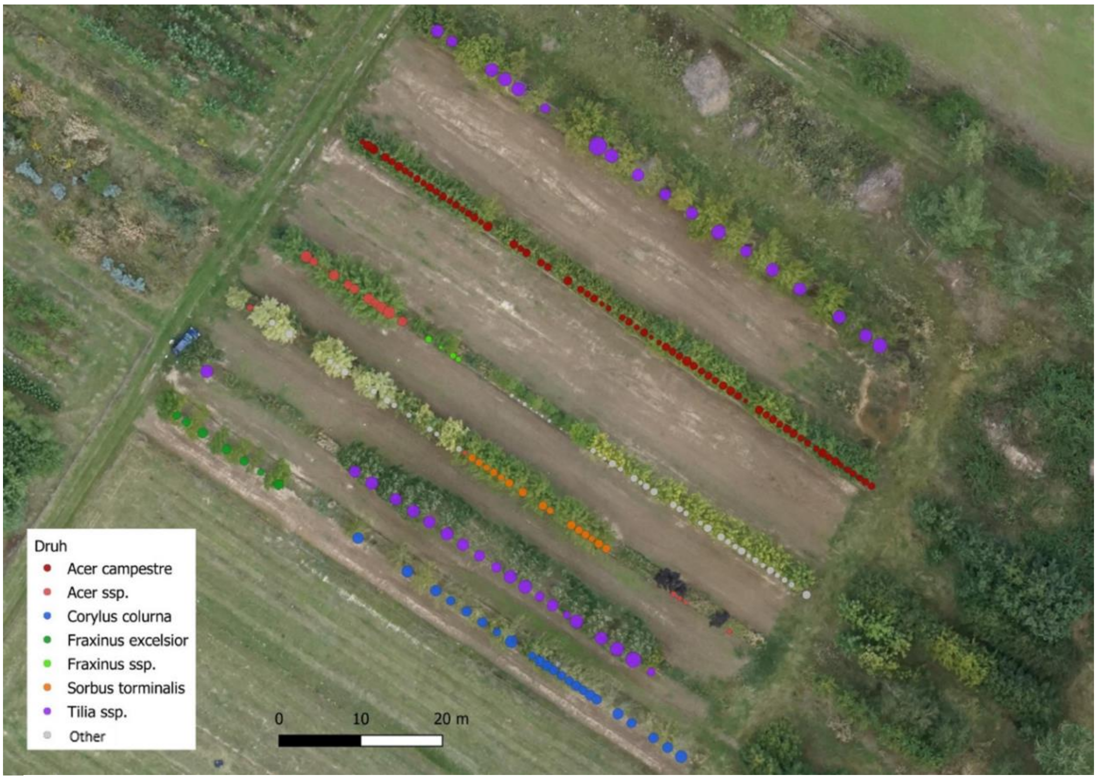
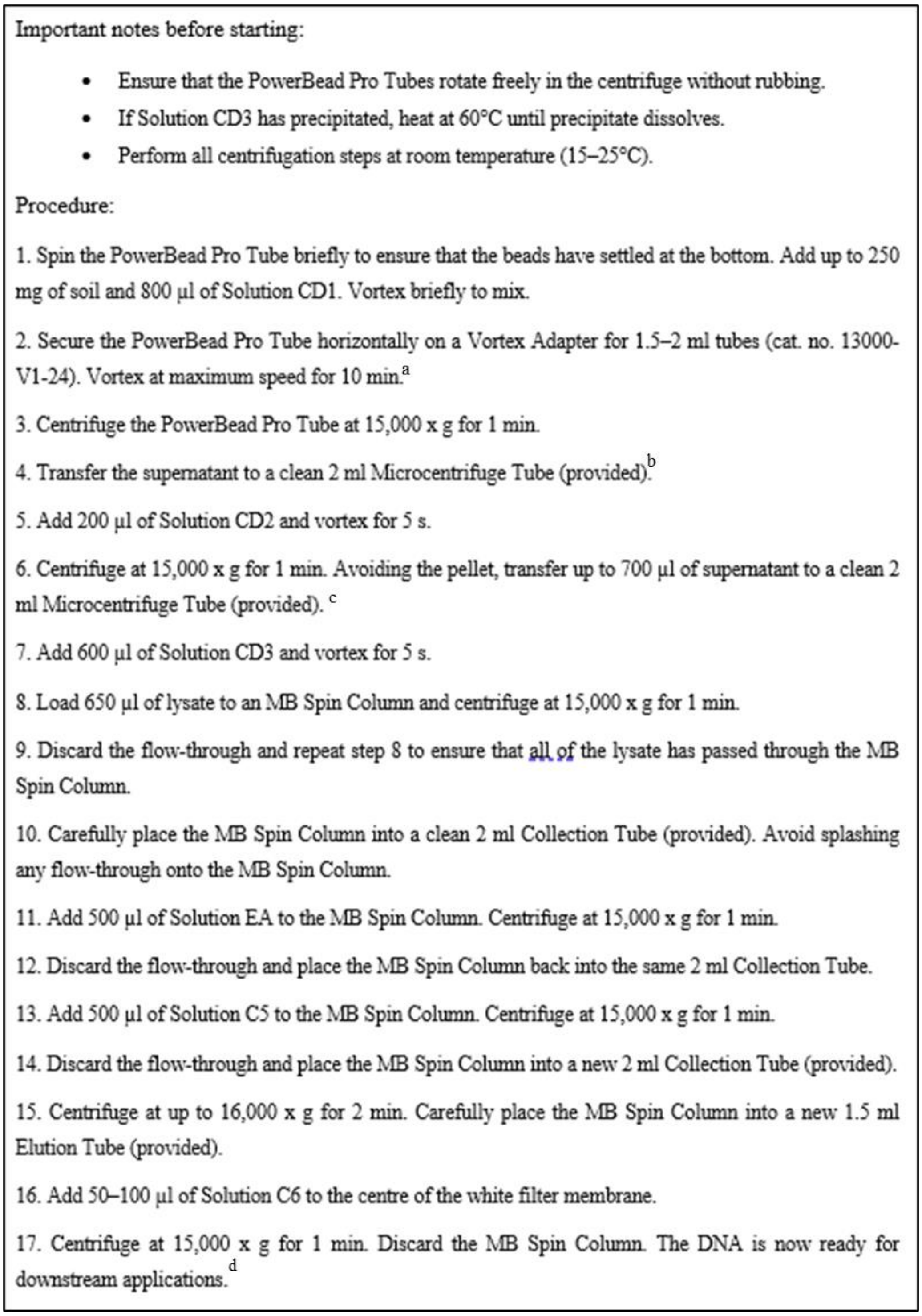


Figure 1: Tree distribution of alley cropping AF system at Michovka, VUKOZ.

Appendix 2

Figure 2: Michovka AF experimental design; alley cropping AF, tree nursery (TN) and neighbouring conventional field (CF).

Appendix 3



Important notes before starting:

- Ensure that the PowerBead Pro Tubes rotate freely in the centrifuge without rubbing.
- If Solution CD3 has precipitated, heat at 60°C until precipitate dissolves.
- Perform all centrifugation steps at room temperature (15–25°C).

Procedure:

1. Spin the PowerBead Pro Tube briefly to ensure that the beads have settled at the bottom. Add up to 250 mg of soil and 800 µl of Solution CD1. Vortex briefly to mix.

2. Secure the PowerBead Pro Tube horizontally on a Vortex Adapter for 1.5–2 ml tubes (cat. no. 13000-V1-24). Vortex at maximum speed for 10 min.[a]

3. Centrifuge the PowerBead Pro Tube at 15,000 x g for 1 min.

4. Transfer the supernatant to a clean 2 ml Microcentrifuge Tube (provided).[b]

5. Add 200 µl of Solution CD2 and vortex for 5 s.

6. Centrifuge at 15,000 x g for 1 min. Avoiding the pellet, transfer up to 700 µl of supernatant to a clean 2 ml Microcentrifuge Tube (provided). [c]

7. Add 600 µl of Solution CD3 and vortex for 5 s.

8. Load 650 µl of lysate to an MB Spin Column and centrifuge at 15,000 x g for 1 min.

9. Discard the flow-through and repeat step 8 to ensure that all of the lysate has passed through the MB Spin Column.

10. Carefully place the MB Spin Column into a clean 2 ml Collection Tube (provided). Avoid splashing any flow-through onto the MB Spin Column.

11. Add 500 µl of Solution EA to the MB Spin Column. Centrifuge at 15,000 x g for 1 min.

12. Discard the flow-through and place the MB Spin Column back into the same 2 ml Collection Tube.

13. Add 500 µl of Solution C5 to the MB Spin Column. Centrifuge at 15,000 x g for 1 min.

14. Discard the flow-through and place the MB Spin Column into a new 2 ml Collection Tube (provided).

15. Centrifuge at up to 16,000 x g for 2 min. Carefully place the MB Spin Column into a new 1.5 ml Elution Tube (provided).

16. Add 50–100 µl of Solution C6 to the centre of the white filter membrane.

17. Centrifuge at 15,000 x g for 1 min. Discard the MB Spin Column. The DNA is now ready for downstream applications.[d]

Figure 3: DNeasy PowerSoil Pro Kit (Qiagen, Germany) protocol for experienced users; different lower case indicates notes.