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DOCTORAL (PhD) DISSERTATION

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DOCTORAL (PhD) DISSERTATION

ASSESSMENT OF SELECTED AGROECOLOGICAL PRACTICES IN SMALL-SCALE MARKET GARDENING

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Моја најдубља захвалност припада менторки, **Др. Аполки**, чија посвећеност није познавала границе: била је уз мене током празника, викенда и у најранијим јутарњим и вечерњим часовима. Бескрајно сам захвална на њеном вођству кроз истраживање и писање, као и на неизмјерној моралној и материјалној подршци која ми је била ослонац. Такође, захваљујем се професорима и колегама са **МАТЕ Универзитета**, као и тиму из **СЗИА Баште** на њиховој великодушној помоћи и сарадњи.

Мојој породици хвала што сте били моје уточиште. Мајци **Милици** и брату **Милораду** хвала што су били уз мене на сваком кораку овог пута. Носила сам с љубављу сјећање на свог **оца**, мог вјечног заштитника, који је у духу био уз мене у сваком тренутку. Такође, дубоко сам захвална својој **„другој породици" у Будимпешти** на охрабрењу и вјери коју су имали у мене.

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ABSTRACT

Sandy soils present one of the greatest challenges to sustainable agriculture due to their low organic matter, weak structure, limited water-holding capacity, and high vulnerability to compaction and nutrient loss. This doctoral research investigates whether conservation tillage (loosening and no-tillage systems) and microbial inoculation can improve soil health, crop performance, and overall system sustainability in a crop rotation, potato (*Solanum tuberosum*), pea (*Pisum sativum*), lettuce (*Lactuca sativa*), implemented in a functioning agroecological market garden in Hungary. The study aimed to (I) evaluate the effects of reduced tillage on soil physical and chemical properties, (II) assess the potential of microbial inoculants to improve nutrient availability and plant performance, (III) examine whether combining these practices leads to synergistic effects, and (IV) generate practical recommendations for sustainable management of soil.

Results demonstrate that while no statistically significant differences were observed in the physical plant parameters across treatments, consistent positive cumulative observations occurred under microbial inoculation (e.g., *Rhizobium* spp., *Pseudomonas* spp., *Bacillus* spp.*)* combined with reduced soil disturbance. A significant difference was identified in the total sugar content of peas, where loosening with microbial inoculation resulted in lower soluble sugar concentration compared to the no-tillage and no microbes, suggesting altered carbon allocation possibly driven by enhanced nitrogen uptake or increased protein synthesis. Soil analysis revealed statistically significant differences in soil plasticity (Arany value) and pH(KCl) after only one year under microbial inoculation, indicating early structural and chemical improvements. Soil resistance and moisture significant differences further supported enhanced soil functioning under reduced tillage.

All soil and plant nutrient parameters remained within agronomically and biologically ranges, consistent with values reported in the literature. The findings confirm that while measurable yield increases may require multiple years, conservation tillage and microbial inoculation contribute to gradual soil regeneration, improved moisture retention, and strengthened soil-plant interactions. This study provides field-based evidence that integrating biological amendments with reduced soil disturbance offers a viable pathway toward agroecological intensification and climate-resilient farming systems.

Keywords: *Conservation tillage; microbial inoculation; PGPR; soil health; agroecology; regenerative agriculture; NDVI; crop rotation; sustainable farming; soil plasticity; plant biochemical response.*

1. INTRODUCTION

1.1. Literature Review

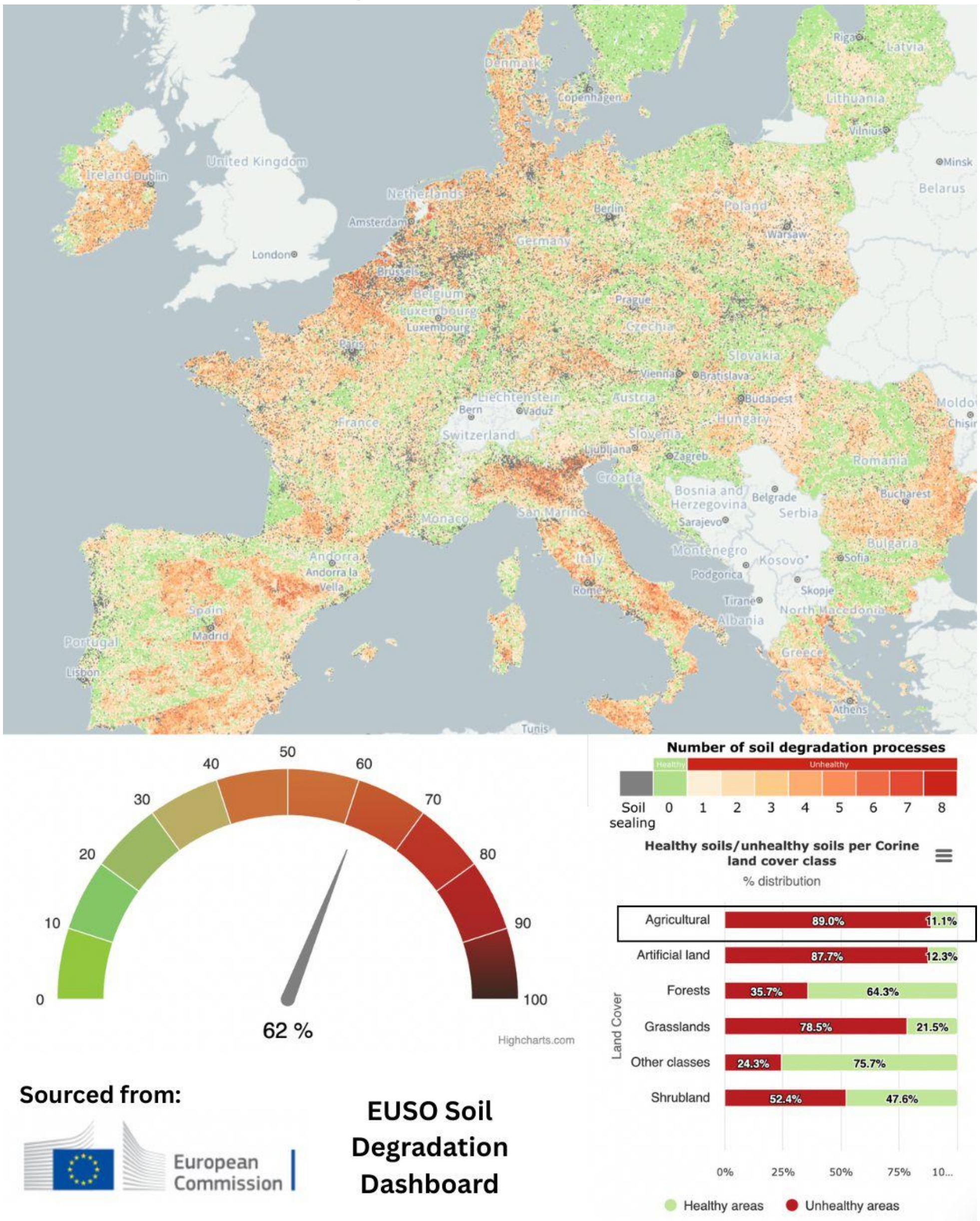
Small-scale farming remains essential to global food systems. It supports the livelihoods of hundreds of millions and plays a vital role in food security, agrobiodiversity, and rural development (Akanmu et al., 2023; Altieri et al., 2012; Dagunga et al., 2023). However, these systems are under increasing pressure from climate change, market fluctuations, land degradation, and social and economic challenges. This situation requires a shift towards more resilient and sustainable agricultural models (Dagunga et al., 2023; Mizik, 2021). Agroecology, which applies ecological principles to agricultural systems, has emerged as a key approach to tackle these challenges. It offers a comprehensive framework that combines environmental, economic, and social aspects of sustainability (Akanmu et al., 2023; Dagunga et al., 2023; Wezel, 2017).

Agroecological practices include a range of techniques, such as crop diversification, intercropping, cover cropping, organic fertilization, integrated pest management, and adding landscape features like hedgerows and buffer strips (Dagunga et al., 2023; Li et al., 2025; Wezel, 2017). These methods aim to improve ecosystem services, promote nutrient cycling, and build resilience against climate and economic challenges (Harkányi and Ujj, 2024). Research has shown that agroecological systems can boost soil productivity, increase biodiversity, and enhance food security for smallholder farmers (Mizik, 2021; Wezel, 2017).

Recent systematic reviews highlight the positive effects of agroecological practices on the resilience and food security of smallholders. This is especially true during events like the COVID-19 pandemic and global market disruptions (Akanmu et al., 2023; Dagunga et al., 2023). In Europe, the adoption of agroecological and technological practices in arable farming varies by region. Smaller, less intensively managed farms are more likely to embrace these practices, though obstacles related to knowledge, policy, and economic incentives still exist (Li et al., 2025). Despite the growing evidence supporting agroecological transitions, there is still lack of a complete understanding of the benefits and trade-offs, particularly regarding productivity, greenhouse gas emissions, and socio-economic outcomes (Li et al., 2025; Mizik, 2021). Additionally, successfully expanding agroecological practices depends on collaborative approaches, inclusive knowledge sharing, and empowering farming communities (Akanmu et al., 2023; Altieri et al., 2012; Batas et al., 2025). Therefore, assessing agroecological practices in small-scale farming and arable land is both timely and crucial for guiding policy, promoting sustainable development, and ensuring resilient food systems for future generations (Ujj et al., 2024).

Recent data from the EU Soil Degradation Dashboard (shown in *Figure 1*) shows that 62% of soils in the European Union are degraded. Agricultural land makes up 89% of this total (http 1, 2026). This poses a serious environmental problem and underscores the urgent need for a major change in land management practices. Key causes of soil degradation include erosion, compaction, and the loss of soil organic matter. These factors together threaten long-term agricultural productivity and ecological health.

Figure 1: Extent and Drivers of Soil Degradation Across the European Union, with Emphasis on Agricultural Land Impact



Source: European Soil Data Centre (ESDAC), EU Soil Degradation Dashboard. Available at: https://esdac.jrc.ec.europa.eu/esdacviewer/euso-dashboard/ (accessed January 2026.)

Addressing these challenges requires a move towards more regenerative farming practices. This change must be backed by strong monitoring systems, science-based actions, and teamwork across different sectors. Currently, there is a great chance of improving agroecological soil management in Europe. By using science-based strategies and encouraging cooperation, there is a possibility to restore soil health and boost the ability of farming systems to capture carbon, helping to meet larger climate resilience goals (Ujj et al., 2020).

With the global population steadily rising, the demand for food and essential nutrients continues to grow. However, this increased demand often puts significant pressure on soil as conventional farming methods can lead to degradation and a decline in soil fertility over time. As mentioned earlier, in recent years, this shift has led to a greater adoption of sustainable farming approaches, with conservation tillage becoming a key strategy for preserving soil and improving productivity (Singh et al., 2020).

Among agroecological practices, conservation tillage has gained wide acceptance (Jug et al., 2025). This is mainly because people recognize the negative effects of conventional tillage methods. Conventional tillage often disrupts the soil a lot. It usually involves using moldboard plows to turn and break up the soil deeply in preparation for planting. While this approach helps in establishing crops quickly, it can cause long-term soil damage (Tobiašová et al., 2023).

Conventional tillage can significantly degrade soil by breaking down its structure, accelerating erosion, and depleting organic matter through increased oxidation. In contrast, conservation tillage methods, such as no-tillage or reduced tillage, help maintain soil integrity, enhance water infiltration, and support the buildup of organic matter (Birkás et al., 2017; Kovács et al., 2023; Lv et al., 2023; Szczepanek et al., 2024). These practices contribute to healthier soils, reduced erosion, and greater long-term sustainability in agriculture. Moreover, the shift toward conservation tillage aligns with broader environmental goals, including climate change mitigation and improved soil management.

Conservation agriculture (CA) encompasses a range of farming practices, including crop rotation, no-tillage, intercropping, and crop-livestock integration, that have been shown to improve soil health and overall soil quality (Lv et al., 2023; Vieira et al., 2021, 2024). Among these, no-tillage (NT) stands out as a key technique, as it directly supports the core principle of conservation agriculture: minimizing soil disturbance (Jug et al., 2025). The effectiveness of these practices is closely tied to the condition of soil, which is heavily influenced by microbial communities. Soil microorganisms play a vital role in maintaining soil productivity by driving nutrient cycling, decomposing organic matter, and supporting soil structure (Vieira et al., 2024). Their activity is fundamental to building and sustaining healthy soils under conservation agriculture systems.

Improving soil productivity is a central aim of agriculture. Traditionally, this has been achieved by using chemical fertilizers to boost nutrient levels (Madawala, 2021). However, the rising costs of these inputs, along with their environmental drawbacks, have presented significant challenges. Excessive reliance on chemical fertilizers can degrade soil, reduce microbial diversity, cause nutrient imbalances, and contribute to water pollution through leaching and runoff. Moreover, their production is energy-intensive and contributes to greenhouse gas emissions and climate change (Alori et al., 2017; Lv et al., 2023; Madawala, 2021; Vieira et al., 2024). In response, conservation agriculture promotes alternatives that reduce or eliminate the need for synthetic fertilizers. One such approach involves the use of beneficial soil microorganisms, such as *Pseudomonas spp*. and *Rhizobium spp.*, which enhance nutrient availability, support root development, and contribute to overall soil health (Kumawat et al., 2022). These microbial-based strategies not only offer a more cost-effective solution for farmers but also align with the principles of sustainability and environmental stewardship (Alori et al., 2017; Kumawat et al., 2022).

Microbial inoculation has gained increasing attention as an effective approach for improving nutrient availability, enhancing soil health, and reducing dependence on synthetic agrochemicals in sustainable agricultural systems (Kour et al., 2020). Despite this progress, organic fertilizers are still largely applied as basal amendments, limiting their capacity to meet crop nutrient requirements throughout the full growth cycle. As the pursuit of higher crop yield and quality intensifies, there is growing interest in nutrient delivery strategies that ensure timely and targeted nutrient supply at different developmental stages (Kour et al., 2020). Solid organic fertilizers often fail to meet nutrient demands during mid- and late-growth phases, when crop requirements are greatest. With advances in modern agricultural technologies, nutrient management is becoming increasingly precise, and the adoption of water-fertilizer integration systems has accelerated the use of liquid formulations as efficient carriers for both nutrients and beneficial microorganisms (Kour et al., 2020).

Within this context, compost tea serves as an illustrative example of a biologically active liquid amendment that supplements microbial inoculation strategies. Produced through the aqueous extraction and fermentation of mature compost, compost tea contains beneficial microorganisms, microbial metabolites, and readily available nutrients that can stimulate soil biological activity and support plant growth (Scheuerell and Mahaffee, 2002). Compared with solid organic fertilizers, compost tea offers rapid nutrient availability and flexible application methods, including foliar sprays for suppressing plant diseases such as mildew, wilt, damping-off, and powdery mildew (Litterick et al., 2004; Morales-Corts et al., 2018), as well as application through drip irrigation or soilless cultivation systems (Ros et al., 2020). From a sustainability perspective, compost tea production aligns with circular economy principles by converting organic waste into value-added inputs and reducing reliance on synthetic fertilizers (De Corato, 2020).

Importantly, the significance of compost tea lies not only in its nutrient content but also in its role as a practical vehicle for microbial inoculation. By delivering diverse and functionally active microbial consortia in a liquid form, compost tea exemplifies how microbial inoculants can be integrated into modern fertilization systems to enhance nutrient use efficiency, strengthen plant-microbe interactions, and support long-term agricultural sustainability (Lalitha, 2017). Thus, while compost tea represents one application approach, the broader objective remains the strategic selection, formulation, and deployment of plant-growth-promoting microbes (PGPM) as central tools for sustainable crop production (Lalitha, 2017).

Plant-growth-promoting microbes (PGPM) play a crucial role in supporting plant development by facilitating key soil and plant processes. They contribute to the decomposition of organic matter and enhance the availability of essential nutrients such as nitrogen, phosphorus, potassium, iron, and magnesium, making them more accessible for plant uptake (Lalitha, 2017). Today, microbial inoculants are widely recognized as an integral component of integrated nutrient management systems. By improving nutrient efficiency and supporting plant health, they contribute significantly to the goals of agriculture and increased crop productivity (Kour et al., 2020).

Soil inoculation refers to the introduction of plant-growth-promoting bacteria (PGPB) into the agricultural environment. These beneficial microbes can be applied directly to seeds, seedling roots, leaves, or soil. Once established in the plant rhizosphere or internal tissues, PGPB enhance plant growth and resilience by improving nutrient uptake, modulating phytohormone levels, and triggering systemic resistance to stress factors (Khoshru et al., 2020). The use of PGPB not only holds promise for increasing crop yields but also for improving the nutritional quality and safety of food products (Asghari et al., 2020). Numerous studies have highlighted the important role of these bacteria in stimulating the production of bioactive compounds, enhancing nutrient availability, and promoting healthy plant growth under a variety of soil and climatic conditions (Asghari et al., 2020; Khoshru et al., 2020; Lalitha, 2017; Alori et al., 2017).

Rhizobium inoculants play a crucial role in promoting nutrient uptake, particularly nitrogen (N), phosphorus (P), and potassium (K), which enhances plant health and ultimately improves crop yields (Basu et al., 2021). What sets *Rhizobium* species apart is their unique ability to form symbiotic relationships with legumes, creating root nodules where they fix atmospheric nitrogen into a form that plants can use (Basu et al., 2021). This process is exclusive to rhizobia and some other specialized bacteria (e.g. *Azospirillum, Azotobacter*), making them invaluable for sustainable agriculture.

In addition to *Rhizobium*, other beneficial bacteria such as *Bacillus*, *Pseudomonas*, and *Ensifer* can colonize legume nodules endophytically (De Meyer et al., 2015). These non-rhizobial bacteria contribute to plant growth through various mechanisms, including the production of plant hormones, phosphate solubilization, and increased enzyme activities like ACC deaminase. While these bacteria support plant growth and stress tolerance, they do not fix nitrogen as efficiently as *Rhizobium* species. The combination of nitrogen-fixing rhizobia and these plant growth-promoting bacteria creates a synergistic effect, enhancing overall soil fertility and crop productivity while promoting persistent and earth-friendly agricultural practices (Kour et al., 2023).

Of all the isolated endophytic bacteria, *Bacillus* and *Pseudomonas* are dominant in nodule tissue; their ubiquity, coupled with their plant-growth-promoting potential and stress tolerance, mark them as useful in sustainable agriculture (Hnini and Aurag, 2024). The occurrence of these bacteria emphasizes the possibility of harnessing beneficial microbial associations as a way of enhancing crop performance, improving soil quality, and reducing dependence on chemical fertilizers.

Immediate actions, such as biofertilization are essential for ensuring crop yields and enhancing environmental sustainability, with legumes providing supplementary ecological advantages that bolster agro-system resilience (Dawa et al., 2014). The anticipated long-term outcomes of this study aim to contribute meaningfully to sustainable agriculture and food security by promoting practices that enhance soil health, optimize resource use, and improve resilience to climate change. By demonstrating the benefits of conservation tillage and microbial inoculants and crop rotation, particularly under Hungarian soil conditions, this research seeks to provide a compelling case for the broader adoption of agroecological practices among small-scale farmers. The combined use of these methods is expected not only to increase crop yields and improve soil structure, but also to enhance carbon sequestration, reduce nutrient leaching, and lower greenhouse gas emissions often associated with conventional farming.

Liu et al. (2023) recommend improving the original soil organic matter content, as well as increasing available nitrogen and phosphorus levels, to maximize the performance of microbial inoculants. They further highlight the importance of maintaining a neutral soil pH (approximately 6-7) to achieve optimal inoculation efficiency (Ahmad et al., 2018; Liu et al., 2023). Collectively, their findings support the strategic use of microbial consortia to enhance biofertilization and bioremediation in living soils and offer valuable perspectives for designing and introducing beneficial microbial communities. The research also emphasizes the advantages of combining multiple inoculants. Specifically, the study confirms a synergistic interaction between *Bacillus* and *Pseudomonas* when applied together as a consortium (Liu et al., 2023). By comparing effect sizes across four treatment groups (consortia containing *Bacillus* alone, *Pseudomonas* alone, *Bacillus* + *Pseudomonas*, and other combinations), the greatest positive impact was observed in the combined treatment. This consortium increased plant growth by 86.6%, demonstrating a markedly enhanced effect (Liu et al., 2023). Both taxa are widely used microbial inoculants in biofertilization and bioremediation, and their complementary functions likely contribute to their strong synergistic performance in soil systems (Ahmad et al., 2018; Liu et al., 2023). Maintaining soil pH within the neutral range (pH 6-8) is particularly important for achieving high inoculation success (Liu et al., 2023). Extreme pH conditions tend to reduce the survival and activity of introduced microbes, thereby limiting the effectiveness of inoculation. A substantial body of research has shown that management practices such as organic fertilization can further enhance crop yields by improving nutrient bioavailability and supporting key functional microbial groups (e.g., phosphorus-solubilizing bacteria) (Ahmad et al., 2018; Liu et al., 2023). Microbial inoculants can be effectively complemented with other soil management practices, such as compost addition or mulching, to amplify their beneficial effects under field conditions (Liu et al., 2023).

Metagenome sequencing has transformed microbial ecology by overcoming the limitations of culture-dependent techniques, allowing direct analysis of genetic material from environmental samples without the need for laboratory cultivation (Nwachukwu and Babalola, 2022). As a culture-independent approach, metagenomics captures a broader spectrum of genetic diversity compared to conventional microbiological methods. Root-associated microbial communities play a crucial role in plant growth, health, and development; therefore, understanding the interactions among soil, plants, and microorganisms is fundamental for improving crop productivity in both rural and urban agricultural systems (Nwachukwu and Babalola, 2022). Comprehensive insights into these microbial communities and their interactions can support the development of sustainable strategies for enhancing plant growth and crop production in both soil-based and soilless agricultural systems (Nwachukwu and Babalola, 2022).

Currently, as mentioned before, the extensive use of chemically synthesized fertilizers and pesticides has resulted in significant negative effects on environmental quality, agricultural ecosystems, and plant health. In response to these challenges, beneficial agricultural microorganisms represent a promising and sustainable alternative for maintaining crop productivity and ecosystem balance (Goel et al., 2017). Despite their abundance and potential, the functional capabilities of many of these microbial communities remain largely unexplored. Considering the vast genetic and metabolic resources harboured by microorganisms, there is a growing need to identify novel genes associated with plant growth-promoting traits that can be developed into effective bioinoculants for sustainable agricultural practices (Gupta et al., 2018). Consequently, the identification and functional characterization of diverse microbial communities have become essential. Metagenome sequencing approaches provide valuable tools for advancing sustainable agriculture by enabling continuous monitoring and assessment of plant growth-promoting microorganisms beyond simple enumeration, with emphasis on their functional roles and interactions within the agroecosystem (Goel et al., 2017).

Crop rotation is widely recognized as a cornerstone of sustainable agricultural practice, particularly in the context of small-scale market gardening (Moldavan et al., 2024; Shah et al., 2021). The use for crop rotation in these systems stems from the need to preserve soil fertility and structure, mitigate cumulative pest and disease pressures, and ensure long-term ecological resilience (Agomoh et al., 2020; Chopin et al., 2026; Merlos and Hijmans, 2020; Moldavan et al., 2024; Shah et al., 2021). The most common rotation is by the plant family (Agomoh et al., 2020; Chopin et al., 2026; Merlos and Hijmans, 2020). The principle of rotation by plant family is essential for maximizing benefits, both for the soil productivity and better yield. It is well established that crops within the same botanical family share similar pest, disease, and nutrient profiles. For example, the *Brassicaceae* (such as cabbages, broccoli, and radishes) are particularly susceptible to clubroot and aphid infestations, while members of the *Solanaceae* (including tomato and potato) are prone to blight and nematode issues (Chopin et al., 2026; Moldavan et al., 2024; Shah et al., 2021). Organizing crop rotation schedules so that members of the same family are not grown in the same bed more than once within a cycle of three to four years reduces the persistence of these risks, ensures a more even demand on macro- and micronutrients, and enables market gardeners to integrate cover and legume crops that further improve soil nitrogen content and organic matter (Agomoh et al., 2020; Chopin et al., 2026; Merlos and Hijmans, 2020).

Rotating potato, peas, and lettuce is beneficial because these crops belong to different plant families, each with distinct nutrient needs and pest susceptibilities, which helps to maintain soil health and reduce disease. Potato (*Solanaceae*) is heavy feeder that deplete soil nutrients, especially nitrogen. Peas (*Fabaceae*), being legumes, fix atmospheric nitrogen into the soil, naturally replenishing it and improving fertility for the following crops. Lettuce (*Asteraceae*), being leafy plant, has different nutrient requirements and pest pressures compared to potato and peas, so planting it after peas and potato disrupts pest and disease cycles and allows the soil to recover (http 2, 2026). This rotation minimizes soil nutrient depletion, reduces pathogen buildup, and supports a balanced, sustainable growing system in small-scale gardens.

Moreover, this research provides practical insights and realistic expectations for farmers considering the transition to more environmentally responsible and agroecological systems. The findings could help reduce dependence on synthetic fertilizers, lower input costs, and contribute to the development of cost-effective, environmentally sound farming practices. Ultimately, this research aims to support a paradigm shift in agricultural management, one that balances productivity with ecological responsibility and equips farmers to face the challenges of a changing climate while ensuring long-term food security.

1.2. Research Background, Problem Statement, and Research Novelty

Research Background

Globally, the increasing pressure on natural resources, climate change, and the environmental costs of industrial agriculture have prompted a shift toward more, ecologically sound farming systems. Central to this transformation is the need to improve soil health, particularly in regions where soil conditions inherently limit agricultural productivity. Although sandy soils are generally considered less fertile, their properties can vary significantly depending on local conditions.

In Hungary, over 1.4 million hectares of land are covered by sandy soils, with the Nyírség region alone comprising more than 400,000 hectares (Aranyos et al., 2016). These soils are formed under conditions of low weathering rates and are typically low in mineral and organic colloids, resulting in poor physical structure and limited nutrient availability (Michéli et al., 2006). In areas without vegetation cover, these soils are prone to wind erosion and surface instability. Conversely, in regions where vegetation has been established and stabilized, organic matter can begin to accumulate, slowly enhancing soil quality. However, under conventional agricultural practices, the full potential of these soils remains largely untapped, and their degradation continues to present ecological and economic challenges.

Among the most pressing limitations of sandy soils is their tendency to compact under mechanical pressure, which reduces pore space, restricts water infiltration and airflow, and inhibits root growth (Aranyos et al., 2016). These issues significantly affect plant health and yield potential, especially in systems that rely on repetitive tillage and external inputs. Therefore, developing low-input, ecologically balanced management practices that can improve soil structure and fertility is of both local and global relevance.

This research emerges in response to the growing demand for improved solutions in soil management, particularly within the framework of agroecology and bio-intensive farming. These approaches aim to reduce dependency on synthetic inputs, enhance biodiversity, and regenerate soil productivity through practices such as composting, crop diversification, reduced tillage, and the use of beneficial soil microbes. By integrating ecological principles into agricultural practice, such methods seek to create systems that are both productive and resilient.

The study was carried out at the SZIA Agroecological Garden. This garden provides ideal research setting for studying land use practices in real-field conditions. Its sandy loam soils, typical of many underperforming agricultural regions in Hungary, present a relevant case for examining the effectiveness of ecological interventions.

A central focus of this research is the development and assessment of a crop rotation strategy combined with agroecological soil management practices. The rotation began with a potato crop planted in the spring-summer of 2023, followed by a legume crop (peas) in 2024, and is scheduled to continue with a leafy vegetable (lettuce) in 2025. This design supports both ecological balance and practical application by simulating conditions in an operating market garden. The study seeks to determine how such interventions affect soil structure, fertility, and overall system productivity, offering insights into scalable models of regenerative agriculture for soils.

Problem Statement

Despite the recognized need for agroecological practices, sandy soils in Hungary remain a limiting factor in achieving consistent, high-yield crop production due to their poor structure, low organic matter, and vulnerability to compaction and erosion. Conventional methods, which often rely heavily on mechanical tillage and chemical fertilizers, tend to exacerbate these problems over time. There is a critical need for practical, scientifically validated strategies that can restore the productivity of soils while minimizing environmental harm.

This research addresses that need by evaluating the combined effects of soil inoculation, reduced tillage, and crop rotation on soil properties and productivity under real market garden conditions. The goal is to contribute knowledge that supports ecologically and economically viable farming practices in Hungary, with relevance beyond the country. In addition, the study aims to enhance the resilience of farming systems by promoting practices that improve soil structure, biodiversity, and nutrient cycling, factors that are essential for adapting to future challenges such as climate variability, resource limitations, and increasing pressures on food production.

Importance and Novelty of Research

While there is increasing interest in agroecological practices, there remains a gap between theoretical frameworks and field-based, replicable methodologies that can be applied under real farming conditions, especially in regions dominated by challenging soil types, which makes this research important.

The novelty of this study lies in its integrated, system-based approach. Rather than isolating single variables in laboratory or pot experiments, the research was designed and implemented in a functional market garden environment. This real-field setting strengthens the applicability and relevance of the results for small-scale growers and market gardeners. By combining soil inoculation, reduced tillage, and carefully planned crop rotation, this study examines how low-input ecological interventions interact to support soil regeneration and enhance overall soil-plant system functioning.

Moreover, the study offers original contributions by evaluating how these practices influence both soil physical properties and crop performance across multiple growing seasons. Through fieldwork, soil analysis, and statistical evaluation, the research not only provides new empirical data on soil management but also contributes to the development of models that can be scaled and adapted to similar agroecological contexts (characterized by limited water availability, or nutrient management challenges).

This research is timely and innovative, bridging the divide between ecological theory and practical implementation. It plays a significant role in advancing the shift towards regenerative and climate-resilient agriculture, especially in areas characterized by naturally less fertile soils.

1.3. Research Objectives and Hypothesis

The primary objective of this research is to examine the individual and combined effects of conservation tillage practices and microbial inoculation on soil physical, chemical, and biological properties, crop yield, and the overall sustainability, including economic aspects, of small-scale agriculture in Hungary. By examining these agroecological practices, this study aims to develop evidence-based guidelines for enhancing soil productivity, reducing reliance on external inputs, and strengthening the economic resilience of small-scale farms. These aims align with Hungary's key environmental and agricultural development goals, which include improving soil quality, promoting resource-efficient and low-input farming systems, increasing climate resilience, supporting farmland biodiversity, and ensuring the long-term viability of small and medium-sized agricultural enterprises.

To achieve this aim, the research sets out to pursue the following specific objectives:

- **Evaluate the effects of minimum tillage and no-tillage systems** on key soil parameters, such as soil organic matter content, moisture retention, nutrient content, and biological activity. This includes assessing how reduced soil disturbance influences physical and chemical soil properties over a single growing season, particularly in the context of sandy loam soils, which are prevalent in many Hungarian farming regions and often suffer from nutrient loss and low water-holding capacity.
- **Assess the agronomic performance and soil-enhancing potential of microbial inoculants,** with an emphasis on plant growth-promoting rhizobacteria (PGPR), such as *Rhizobium spp., Ensifer spp., Pseudomonas spp.,* and *Bacillus spp*. This objective involves measuring their effect on soil nutrient availability (e.g., nitrogen, phosphorus), microbial activity, and enzymatic processes, as well as their influence on crop performance, including size, yield, and quality. The research aims to determine whether these bio-based inputs can serve as viable alternatives or supplements to chemical fertilizers, particularly under the economic and environmental conditions faced by smallholder farmers.
- **Examine the synergistic interactions between conservation tillage and microbial inoculation,** investigating whether the combined application of these practices produces additive or multiplicative benefits in terms of soil properties, crop yield, nutrient cycling, and moisture dynamics. This includes identifying any mechanisms through which microbial inoculants may perform more effectively in the improved microenvironment

created by reduced tillage systems, potentially enhancing microbial colonization and activity.

- **Provide evidence-based, context-specific recommendations to farmers, policymakers, and agricultural stakeholders on the practical benefits and potential limitations of adopting conservation tillage and microbial inoculants.** This objective aims to clarify the realistic outcomes that can be expected within a one-year observation period, focusing on short-term effects on soil health, nutrient availability, moisture retention, yield and quality of the final product. The research considers how these practices align with existing agroecological support programs, such as the Hungarian Agroecology Program and the EU's Farm to Fork strategy, offering guidance on how such approaches can contribute to long-term goals including improved soil conservation, greater resilience to climate change, and enhanced sustainability in small-scale agricultural systems.

By fulfilling these research objectives, the research aims to contribute meaningfully to the development of agricultural systems that enhance soil properties, optimize resource use, and improve the livelihoods of small-scale farmers. It also supports broader policy goals related to food security, environmental responsibility, and climate change mitigation, both within Hungary and in the wider European context.

This study is guided by the hypothesis that conservation tillage (loosening and no-till) will improve soil structure and increase plant yield over multiple growing seasons. Furthermore, it is hypothesized that inoculation with beneficial bacteria will enhance nutrient availability, stimulate plant growth, and lead to higher yield and overall contribute to soil and plant quality. The study also is guided by that combining conservation tillage with microbial inoculation will generate synergistic effects, leading to greater improvements in both soil health and crop performance than either practice could achieve on its own. In this context, soil health refers specifically to enhancements in soil physical structure, nutrient availability, and biological activity, attributes that collectively support improved crop growth and overall ecosystem functioning.

2. MATERIALS AND METHODS

2.1. Materials

This research used a variety of materials for field cultivation and laboratory analysis. It focused mainly on potato, pea, and lettuce crops, as well as related soil properties. The goal was to evaluate soil conditions, crop growth, and the microbial communities that are associated with them. To accomplish this, the study combined agroecological farming methods with modern analytical techniques. This approach provided a thorough understanding of the interactions between crops and their growing environment.

Overview of the General Field and Cultivation Materials

For all plant cultivation, the following tools were used during planting, growth, and harvesting phases: trowels, measuring tapes, levelling tools, weed extraction tools, buckets, harvesting tools, containers, bins, callipers, weighing scales, and broad forks for loosening the soil.

To characterize the site at the start of the study, microbial analysis was performed utilizing paid service by the Xenovea Szolgáltató Kft. Metagenomic DNA was extracted from 50-100 mg of soil using the ZymoBIOMICS 96 MagBead DNA Kit (Zymo Research), following the manufacturer's protocol. Sequencing data (>20 million reads per sample) were demultiplexed and adapter-trimmed with NovaSeq Control Software. Base trimming (at both 3' and 5' ends) and quality filtering (Q<30 and reads <100 bp) were performed using the FastQ Toolkit (Illumina). Taxonomic profiling was completed using the DRAGEN Metagenomics Pipeline 3.5.13 (Illumina), enabling a high-resolution view of soil microbial communities.

For soil penetration resistance, a penetrometer was employed to assess compaction levels across plots. Soil moisture was evaluated using samples collected with an auger, followed by oven drying in the university laboratory to determine gravimetric moisture content. Soil infiltration rate was assessed using a single-ring infiltrometer: the metal ring was inserted into the soil, and a known volume of water was poured into the ring to measure infiltration time, helping characterize soil permeability.

The NDVI measurements were carried out using drone technology, in collaboration with colleagues from the HUN-REN-MATE Agroecology Research Group, Department of Plant Physiology and Plant Ecology, Institute of Crop Production Sciences.

Table 1 provides comprehensive overview of the materials used over the years, detailing the plant species cultivated and the associated experimental components.

Table 1: Summary of Experimental Setup, Biological Inoculants, and Analytical Equipment Used for Soil and Plant Sample Analyses (2023-2025)

Year	Crop Scientific Name	Planting Material	Soil Bacteria (Phylazonit Inoculant Strains)	Soil Sample Analysis Equipment	Plant Sample Analysis Equipment
2023 (I year)	Potato (*Solanum tuberosum*)	Organic seeds of potato (type *Desiree*)	*Pseudomonas putida, Azotobacter chroococcum, Bacillus circulans, Bacillus megaterium* L.	**Sample Preparation:** Drying cabinet - Memmert UFE (Germany) Soil grinder - Retsch SK 100 (Germany) Muffle furnace - Nabertherm (Germany) Sieve shaker - Retsch AS 200 (Germany) Calcimeter - Scheibler (Germany) **Basic Measurements:** Analytical balance - Ohaus Adventurer Pro (USA) Tare balance - Ohaus Explorer Pro (USA) pH and conductivity meter - Thermo Scientific Orion 5 (USA) **Spectroscopic and Elemental Analysis:** UV–Vis spectrophotometer - Shimadzu UV-1800 (Japan) Flow injection analyzer - FOSS FIAstar 5000 (Denmark) ICP-OES - PerkinElmer Avio 220 Max (USA)	**Sample Preparation:** Drying cabinet - Thermocenter TC40 (SalvisLab / SalvisLab, Switzerland) Water bath - Memmert WNB14 (Germany) Shaking water bath - Memmert WNB 22 (Germany) Shaker - Edmund Bühler KS-15 (Germany) Hot plate - Harry Gestigkeit HD2 (Germany) Hot plate - Ceran 500 (Germany) Muffle furnace - Nabertherm P320 (Germany) Muffle furnace - Nabertherm LT24/11/P330 (Germany) Plant grinder - IKA M20 (Germany) **Basic Measurements:** Analytical balance - Prescisa 180A (Switzerland) Analytical balance - Scaltec SPB31 (USA) Analytical balances - OHAUS / Ohaus Adventurer Pro (USA) Scale - Ohaus Explorer Pro (USA) pH and conductivity meter - Thermo Scientific Orion 5 (USA) **Chemical and Protein Analysis:** Auto digestion block - Foss Tecator Digestor (Denmark) Scrubber - Foss Tecator (Denmark) Protein analyzer - Foss Kjeltec 8400 (Denmark) **Spectroscopic and Elemental Analysis:** UV–Vis spectrophotometer - Shimadzu UV-1800 (Japan) HPLC - Shimadzu UFLC (Japan) Flow injection analyzer - FOSS FIAstar 5000 (Denmark) ICP-OES - PerkinElmer Avio 220 Max (USA) Atomic absorption spectrophotometer - Solaar M6 (USA) **Optical Analysis:** Polarimeter - ATAGO AP300 (Japan)
2024 (II year)	Peas (*Pisum sativum*)	Organic pea seeds supplied by Rédei Kertimag Zrt. (Hungary)	*Rhizobium leguminosarum, Ensifer kummerowiae, Pseudomonas putida, Pseudomonas fluorescens, Bacillus megaterium,* and *Bacillus subtilis*. L		
2025 (III year)	Lettuce (*Lactuca sativa*)	Organic lettuce seeds provided by Garafarm Trade Kft. (Hungary) Transplanting in March 2025; direct field planting in April 2025	*Pseudomonas putida, Pseudomonas fluorescens, Bacillus megaterium,* and *Bacillus subtilis.* L		

All measurements were conducted in MATE University's accredited laboratory (MATE Agrártudományi Vizsgálólaboratórium HUN.)

Source: Own work

Statistical Analysis: To introduce a statistical dimension to the results, One-way Analysis of Variance (ANOVA) was performed using the R programming language, allowing for the calculation of p-values and visualization of differences through box plots.

2.2. Methodology

This research was initiated through an experimental trial conducted in a controlled garden environment designed to simulate real-field agricultural conditions while allowing for precise management of key variables. The study focused on three core agronomic practices: soil inoculation, crop rotation, and minimal soil tillage (loosening and no-till).

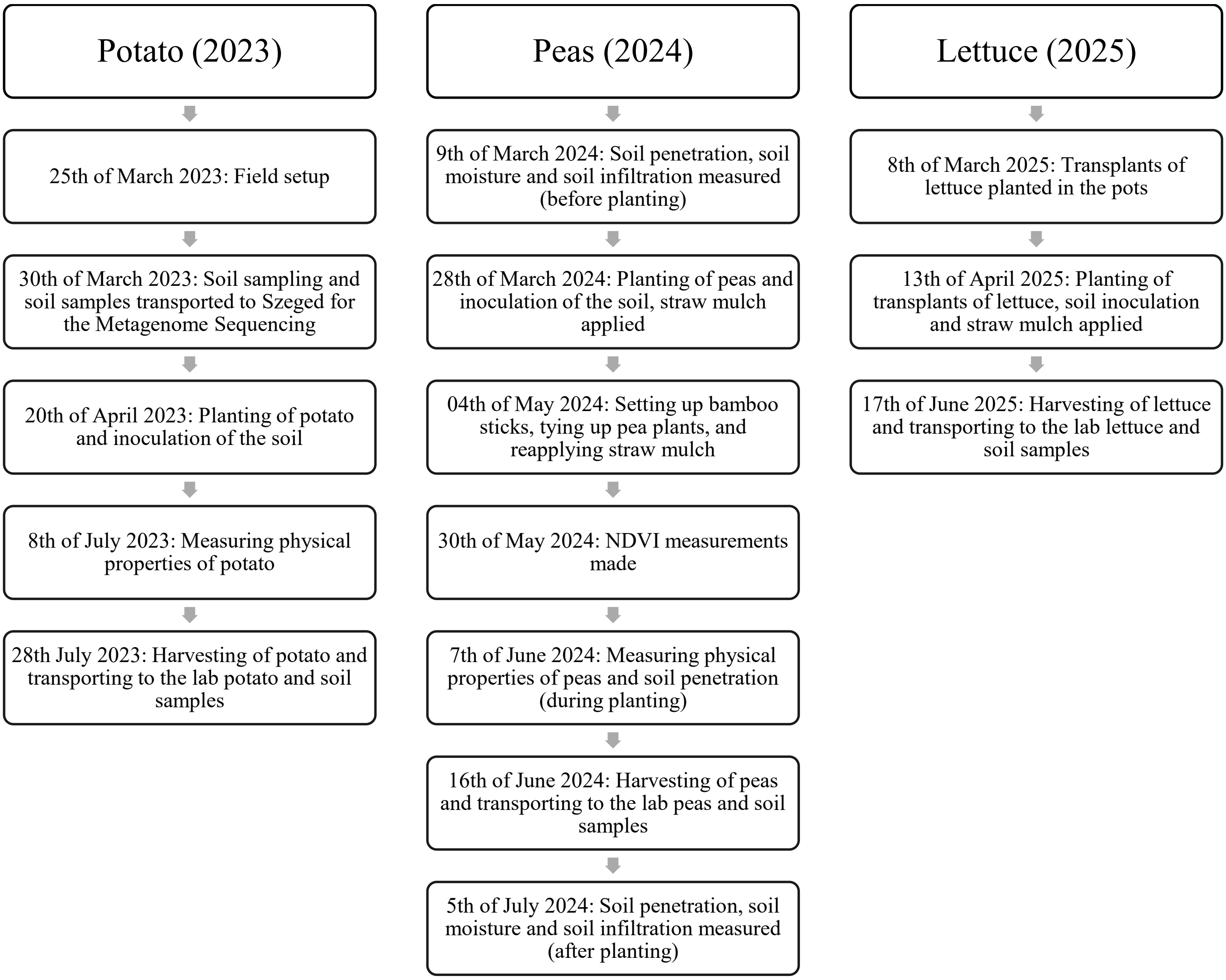
Each of these practices was incorporated with specific objectives: **(I) soil inoculation** aimed to provide a cost-effective alternative to conventional chemical fertilizers by introducing beneficial soil microorganisms; **(II) crop rotation** was implemented to enhance both ecological balance and economic efficiency, adhering to the principles of bio-intensive gardening; and deep tillage was replaced by alternative **(III) soil loosening and no-tillage** to preserve soil structure and promote long-term soil health.

The experimental site was located at the SZIA Agroecological Garden at the Hungarian University of Agriculture and Life Sciences (MATE) in Gödöllő, Hungary (GPS coordinates: 47.5941° N, 19.3593° E). Established in 2019 and relocated to the university premises in 2021, this garden functions as a valuable platform for agroecological research, education, and community engagement. The garden also serves a social role, hosting educational programs, volunteer initiatives, and activities aimed at individuals with disabilities and marginalized groups, thereby fostering inclusivity alongside sustainable environmental management.

Although the experiment was conducted on a small-plot scale, it was designed within a fully operational market garden applying bio-intensive crop rotation practices. This setting ensured the study reflected practical agricultural production conditions and eliminated the need for pot experiments, thereby increasing the relevance and scalability of the results to broader agricultural contexts. Preparatory work for the cultivation of experimental crops was carried out in advance to ensure systematic implementation.

Preparation for potato cultivation started in 2023, and planting took place in April 2023. Soil inoculants were applied during planting to improve soil microbial activity. The potato crop was harvested in late July 2023, completing the initial phase of the experiment. The research then moved on to growing peas, with preparatory work and planting finished by late March 2024. Soil inoculation continued throughout the pea growing period to support soil microbial health. Peas were harvested in mid-June 2024. After that, lettuce cultivation began in early March 2025, with soil inoculants applied again to further improve soil conditions. The final harvest, lettuce, was completed in mid-June 2025, wrapping up the field experiment cycle. The timeline of field activities is presented in *Figure 2.*

Figure 2: Detailed timeline of field activities and sampling for potato, peas, and lettuce throughout the study period



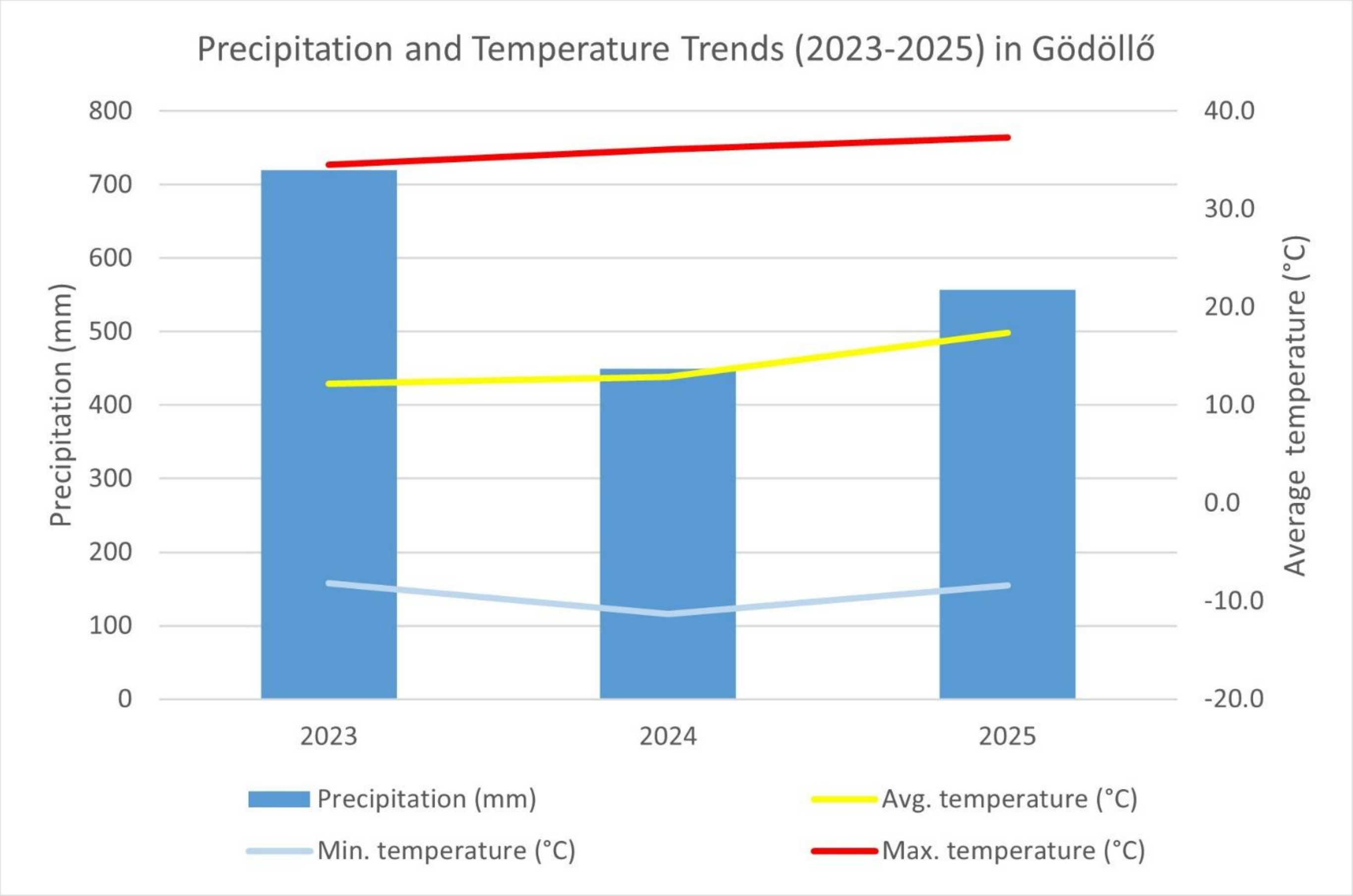
Source: Own work

Straw mulch during peas and lettuce growing was applied to the soil surface. This practice was employed primarily to suppress weed growth, conserve soil moisture, and regulate soil temperature, thereby creating more favourable conditions for crop development and promoting overall soil health.

Data collection was conducted systematically throughout the experimental period. Crop samples were collected to measure physical parameters such as size, weight, and other morphological characteristics. Chemical analyses of the crop tissues were performed in the laboratory to assess nutrient content and quality. Simultaneously, soil samples were taken and analysed for a range of physicochemical properties to evaluate soil fertility and microbial activity.

To characterize the field conditions during the study period, *Figure 3* presents the annual precipitation and temperature data for 2023, 2024, and 2025, providing an overview of the prevailing climatic conditions. The meteorological dataset was obtained from Waltner et al. (2025). For the purposes of this study, the 10-minute meteorological observations were aggregated and extracted as needed to produce the annual precipitation totals and average temperature values displayed in *Figure 3*.

Figure 3: Precipitation and Temperature Trends (2023-2025) in Gödöllő



Source: Own work

The collected data were visually represented using box plot diagrams, providing an overview of variability and distribution. Statistical analysis was conducted using one-way Analysis of Variance (ANOVA) in the R programming environment to determine the significance of differences observed between treatment groups and to draw conclusions regarding the effects of the implemented practices.

This methodology integrates agronomic techniques within a practical, market-oriented setting, aiming to balance ecological benefits with economic viability. The detailed procedures for experimental design, sample collection, laboratory analysis, and statistical evaluation are presented in the subsequent sections to ensure reproducibility and transparency.

2.2.1. Experimental Field Design and Setup at SZIA Agroecological Garden, MATE, Gödöllő

The practical phase of this research was conducted at the SZIA Agroecological Garden, located at the Hungarian University of Agriculture and Life Sciences (MATE) in Gödöllő, Hungary. The garden provided an ideal environment for controlled experimentation, enabling the investigation of sustainable soil management practices under realistic field conditions.

In April 2023, the garden was subdivided into 12 distinct plots, each measuring 80 cm in width and 300 cm in length (80 cm x 300 cm). These dimensions were standardized across all plots to facilitate consistent and accurate data collection. The plots were carefully measured and spaced apart by additional beds, serving as physical barriers to prevent any potential cross-contamination between different microbial treatments. This spatial separation was critical for maintaining the integrity of the experimental design and minimizing treatment interference.

The experiment employed a randomized complete block design (RCBD) with a split-plot arrangement, replicated three times to ensure statistical robustness and account for environmental variability, such as soil fertility gradients and moisture differences. The design allowed precise assessment of the impacts of tillage and microbial inoculation on soil dynamics and crop growth.

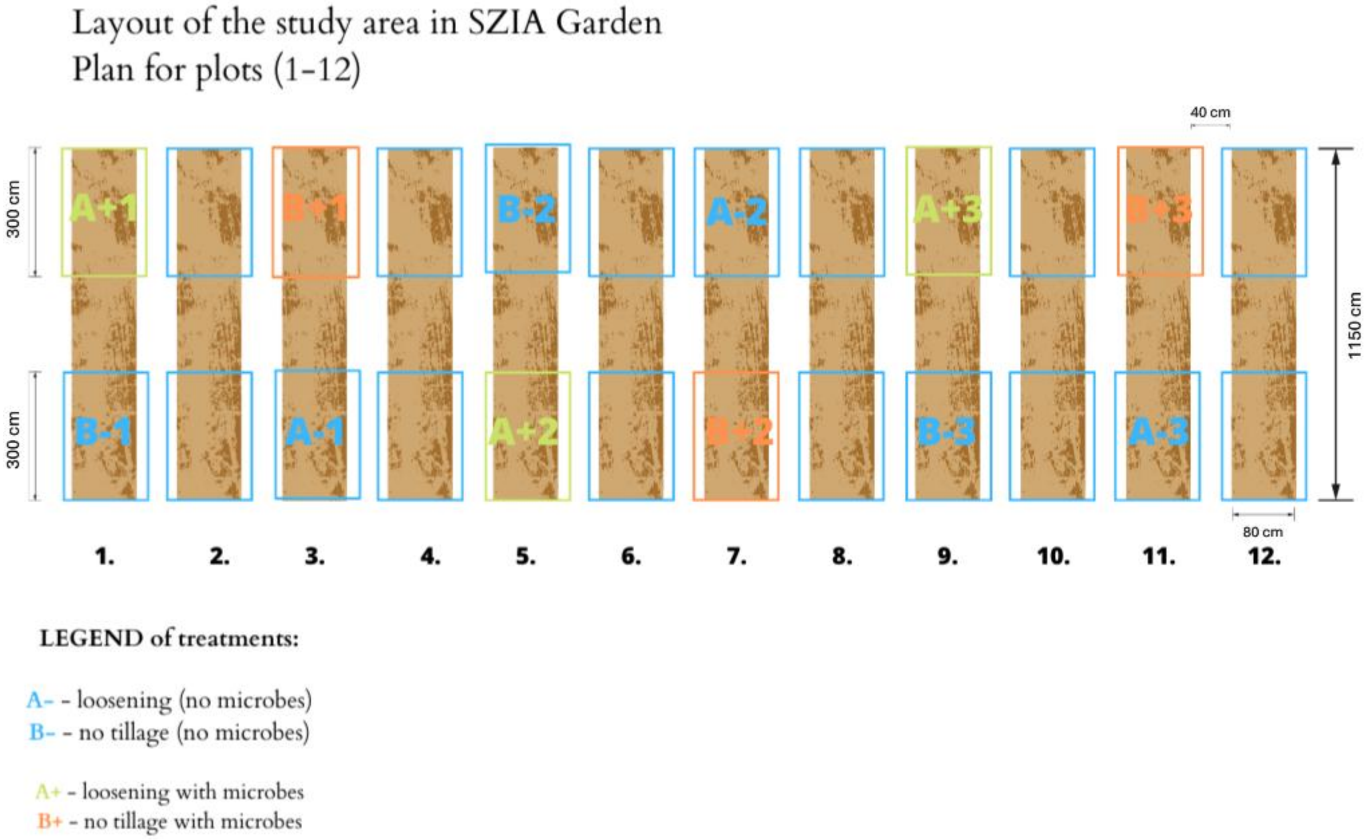
Four treatment groups were established, each replicated three times, resulting in a total of twelve experimental plots:

- **A- (Loosening without Microbes):** Soil was subjected to loosening techniques without the addition of microbial inoculants.
- **B- (No-Tillage without Microbes):** Soil remained undisturbed without microbial inoculation.
- **A+ (Loosening with Microbes):** Loosening was combined with the introduction of beneficial microbial agents.

- **B+ (No-Tillage with Microbes):** No-tillage practices were applied alongside microbial inoculants to evaluate their combined effect on soil properties.

A visual representation of the experimental layout is provided in *Figure 4* is offering an overview of the design and spatial organization of the study plots.

Figure 4: Experimental layout of the field plots used during the study



Source: Own work

The experiment started with the careful application of the assigned tillage treatments for each plot. All procedures were performed with attention to detail to ensure reliable results. The main goal was to compare the effects of no-tillage and soil loosening, both with and without microbial inoculants, on soil properties and crop performance.

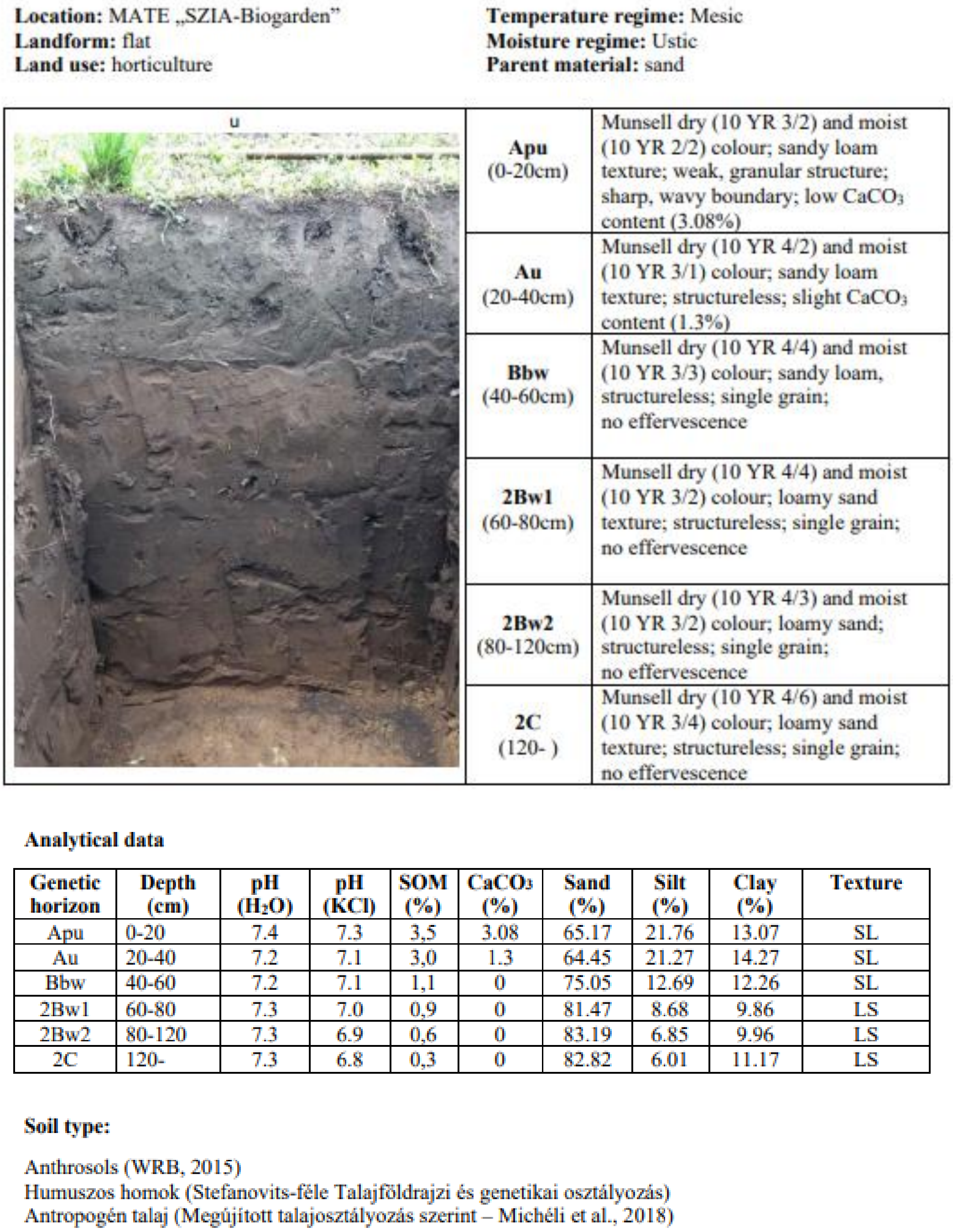
Figure 5 presents a current photograph of the SZIA Agroecological Garden, illustrating the live conditions of the experimental field throughout the research period.

Figure 5: Recent view of the SZIA Agroecological Garden, showing the condition of the experimental field

Source: Own work

Figure 6 provides an in-depth description of the study area, highlighting the soil horizon profile and offering significant context regarding the soil-related elements of the experiment.

Figure 6: Description and profile of soil horizons at the experimental site



Location: MATE „SZIA-Biogarden”
Landform: flat
Land use: horticulture

Temperature regime: Mesic
Moisture regime: Ustic
Parent material: sand

Apu (0-20cm)	Munsell dry (10 YR 3/2) and moist (10 YR 2/2) colour; sandy loam texture; weak, granular structure; sharp, wavy boundary; low $CaCO_3$ content (3.08%)
Au (20-40cm)	Munsell dry (10 YR 4/2) and moist (10 YR 3/1) colour; sandy loam texture; structureless; slight $CaCO_3$ content (1.3%)
Bbw (40-60cm)	Munsell dry (10 YR 4/4) and moist (10 YR 3/3) colour; sandy loam, structureless; single grain; no effervescence
2Bw1 (60-80cm)	Munsell dry (10 YR 4/4) and moist (10 YR 3/2) colour; loamy sand texture; structureless; single grain; no effervescence
2Bw2 (80-120cm)	Munsell dry (10 YR 4/3) and moist (10 YR 3/2) colour; loamy sand; structureless; single grain; no effervescence
2C (120-)	Munsell dry (10 YR 4/6) and moist (10 YR 3/4) colour; loamy sand texture; structureless; single grain; no effervescence

Analytical data

Genetic horizon	Depth (cm)	pH (H_2O)	pH (KCl)	SOM (%)	$CaCO_3$ (%)	Sand (%)	Silt (%)	Clay (%)	Texture
Apu	0-20	7.4	7.3	3,5	3.08	65.17	21.76	13.07	SL
Au	20-40	7.2	7.1	3,0	1.3	64.45	21.27	14.27	SL
Bbw	40-60	7.2	7.1	1,1	0	75.05	12.69	12.26	SL
2Bw1	60-80	7.3	7.0	0,9	0	81.47	8.68	9.86	LS
2Bw2	80-120	7.3	6.9	0,6	0	83.19	6.85	9.96	LS
2C	120-	7.3	6.8	0,3	0	82.82	6.01	11.17	LS

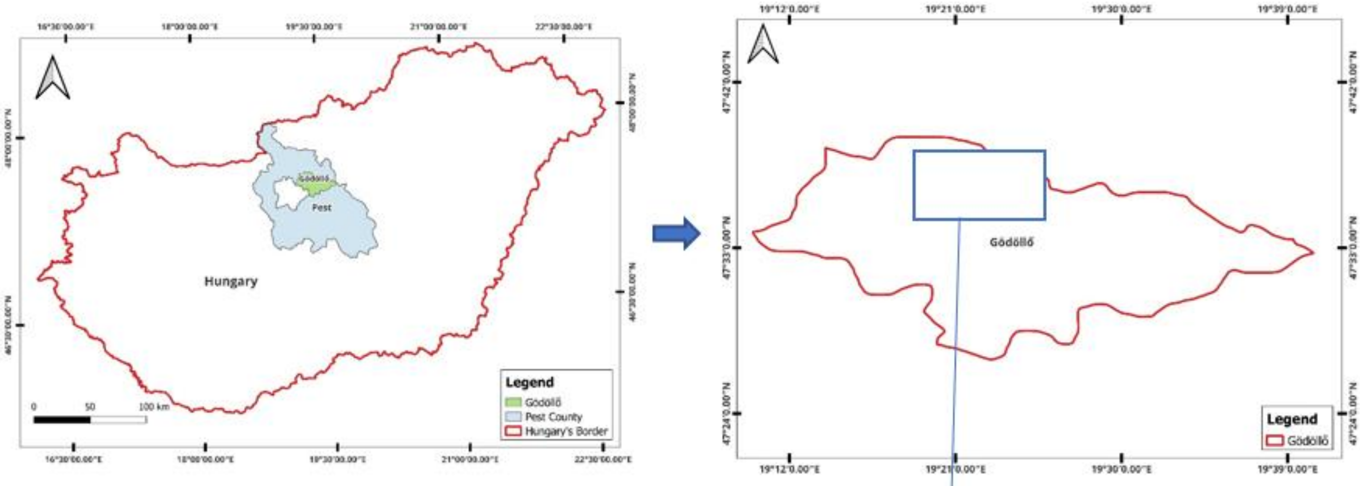
Soil type:

Anthrosols (WRB, 2015)
Humuszos homok (Stefanovits-féle Talajföldrajzi és genetikai osztályozás)
Antropogén talaj (Megújított talajosztályozás szerint – Michéli et al., 2018)

**SL: sandy loam*
**LS: loamy sand*
Source: MATE Soil Research Team

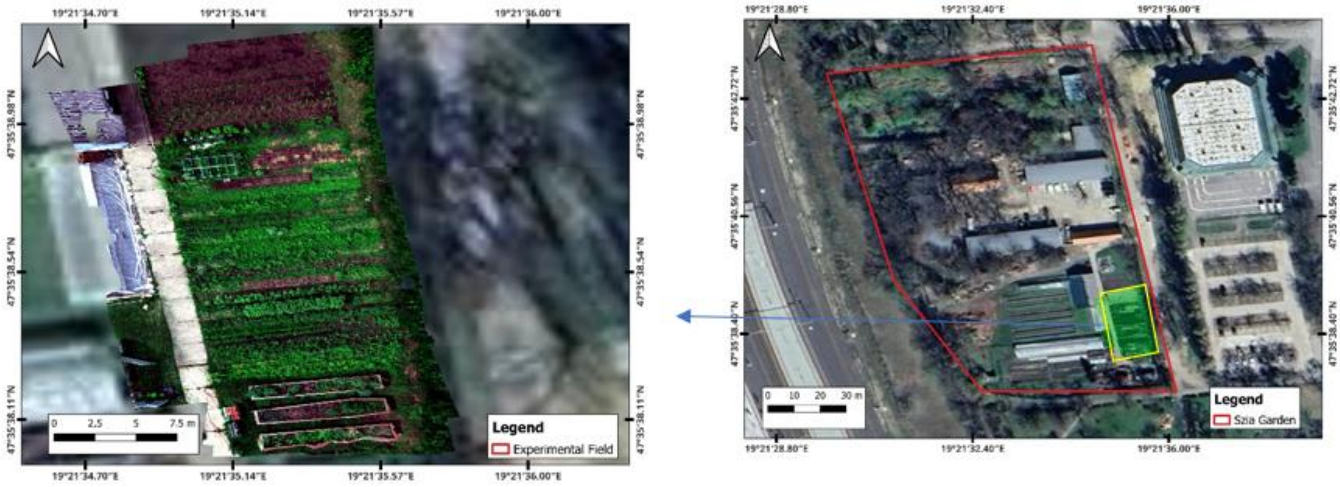
Figure 7 displays the garden’s layout, created using QGIS and images from Google Earth.

Figure 7: Overview of the research site across four spatial scales



a. Hungary, showing Pest County and the City of Gödöllő

b. Gödöllő city



c. Precise Experimental Plots within SZIA Garden

d. SZIA Garden Area in Gödöllő

(a) Hungary with Pest County highlighted and the location of Gödöllő; (b) Administrative placement of Gödöllő within Pest County; (c) Detailed view of the specific experimental plot where the research was conducted; (d) Research area showing greenhouses and field structures with the experimental field
Source: Own work

2.2.2. Measurement of Physical Properties of Plants

The physical properties of the cultivated plants were assessed annually, such as plant height, number of plants per plot, and general morphological characteristics. Plant height was measured using a measuring tape, while plant counts were conducted manually along predetermined plot transects. Where applicable, total above-ground biomass was determined by harvesting representative plants and weighing them using a laboratory precision scale.

Weed Assessment: In Year I, weed biomass was quantified following Weed Sampling Tutorial (http 3, 2026). All weed biomass (with roots) within each designated plot was harvested and weighed using a precision weighing scale to determine total weed biomass. In Years II and III, weed biomass was not measured, as a straw mulch layer was applied to all plots, reducing weed appearance. The absence of weed measurements in these years does not compromise the integrity of the experiment, as weed suppression through mulching represents an intentional and uniform management intervention rather than an uncontrolled variable. Since the mulch was applied consistently across all treatments, it does not introduce bias or confounding differences between experimental groups.

Overall, the assessment of plant physical properties relied primarily on direct measurement tools, measuring tapes for plant height, manual plant counts for density, and precision weigh scales for biomass determination.

2.2.3. Application of Soil Inoculants in Crop Cultivation

The application of microbial soil inoculants was a key component of this research, aimed at enhancing nutrient availability, and crop growth across the different plantings: potato, pea, and lettuce. The inoculants, donated by the Hungarian Phylazonit company, used in this study contained carefully selected strains of beneficial bacteria indigenous to the Carpathian Basin, ensuring ecological compatibility and minimizing disruption to native soil microbiota.

Potato Cultivation: During the potato planting phase, the **soil inoculant**, composed by the mixture of beneficial bacteria, was applied to improve nutrient cycling, plant growth, and soil quality. The inoculant comprised bacterial strains including *Pseudomonas putida*, *Azotobacter chroococcum*, *Bacillus circulans*, and *Bacillus megaterium L.*, formulated in an optimized ratio with a high concentration of 10^9 Colony-Forming Units (CFUs) per cm^3. A nutrient medium was included to support bacterial viability and activity upon application.

Following the manufacturer’s protocol the inoculant was incorporated directly into the planting holes during seedbed preparation, to a depth corresponding to the potato sowing depth (20 cm). This precise placement ensured close contact between the bacteria and the developing roots, facilitating improved nutrient uptake and root colonization. The nitrogen-fixing bacteria within the inoculant played a critical role in converting atmospheric nitrogen into forms accessible to the potato plants, while phosphorus-mobilizing bacteria released essential nutrients necessary for early plant development. Additionally, secondary metabolites such as organic acids produced by these bacteria stimulated vital plant-soil metabolic processes, promoting a nutrient-rich rhizosphere environment.

The inoculant application was performed using a 5 L inoculant-water mixture, with 15 mL of the suspension applied per 2.4 m² plot, ensuring targeted and efficient inoculation. This precise dosage ensured effective colonization without waste. *Figure 8* illustrates the application process of microbial inoculants during potato planting.

Figure 8: Application of Microbial Soil Inoculants in Potato Planting (I year-2023)

Source: Own work

Pea Cultivation: For the pea planting, the study employed application of microbial inoculants to assess their effect on legume growth and soil improvement. The bacterial composition for peas included *Rhizobium leguminosarum*, *Ensifer kummerowiae*, *Pseudomonas putida*, *Pseudomonas fluorescens*, *Bacillus megaterium*, and *Bacillus subtilis*. Symbiotic nitrogen-fixing strains (*Rhizobium leguminosarum* and *Ensifer kummerowiae*) constituted approximately 40% of the mixture, while the remaining 60% consisted of strains known for their soil regenerative capabilities, contributing to nutrient solubilization and pathogen suppression (see *Table 2* for details).

Table 2: Composition of soil amendment mixture (Peas II year-2024)

Category	Strain	Function
Symbiotic Nitrogen Fixers (40%)	*Rhizobium leguminosarum*	Forms nitrogen-fixing nodules on legumes, enhancing soil fertility and reducing reliance on synthetic fertilizers
Symbiotic Nitrogen Fixers (40%)	*Ensifer kummerowiae*	Nitrogen-fixing bacterium that improves nitrogen availability through its symbiotic relationship with legumes
Soil-Regenerating Strains (60%)	*Pseudomonas putida*	Produces siderophores for enhanced iron availability and degrades organic compounds, promoting soil health
Soil-Regenerating Strains (60%)	*Pseudomonas fluorescens*	Biocontrol agent against pathogens and phosphate solubilizer, promoting plant health
Soil-Regenerating Strains (60%)	*Bacillus megaterium*	Phosphate solubilizer that enhances phosphorus availability and produces growth-promoting substances
Soil-Regenerating Strains (60%)	*Bacillus subtilis*	Suppresses pathogens and improves soil structure via exopolysaccharide production

Source: Phylazonit Catalogue

In accordance with guidelines, the inoculant was mixed into the planting holes during seedbed preparation, at the depth appropriate for pea sowing. The nitrogen-fixing bacteria facilitated biological nitrogen fixation, enriching soil nitrogen levels critical for legume development. Other bacteria enhanced nutrient bioavailability, supported root growth, and increased plant resilience against environmental stressors and diseases. The inoculant application was performed using a 5 L inoculant-water mixture, with 15 mL of the suspension applied per 2.4 m^2 plot, ensuring targeted and efficient inoculation.

Lettuce Cultivation: For the lettuce transplanted in early March 2025, soil inoculation followed similar protocols as the previous crops to maintain and enhance soil microbial activity. Lettuce planting with transplants and inoculation has been shown in *Figure 9*. The same bacterial consortia, selected for their plant growth-promoting and soil benefits, were applied during planting by incorporating the inoculant into the planting rows, ensuring effective root-zone colonization. Application rates and preparation mirrored those used for potato and peas to ensure consistency and optimize microbial efficacy. For lettuce cultivation, the "General Soil Regenerator" was used as the microbial inoculant. This bio-preparation contains a consortium of beneficial bacterial strains, including *Pseudomonas putida*, *Pseudomonas fluorescens*, *Bacillus megaterium*, and *Bacillus subtilis*. These strains are known for their plant growth-promoting properties, such as enhancing nutrient availability and stimulating root development, and improving plant resilience to stress. The inoculant has a germ count of 10^9 colony-forming units (CFU) per cm^3, formulated in a nutrient-rich medium to support bacterial viability and activity in the soil.

Figure 9: Application of Microbial Soil Inoculants in Lettuce Planting with Transplants (III year-2025)

Source: Own work

2.2.4. Soil Sampling Procedure Following Crop Harvests

Soil sampling was conducted after the harvest of potato, peas, and lettuce from the 12 experimental plots, in accordance with the objectives of SOILGUARD project and following the Soil Sampling Guidelines under Grant Agreement No. 101000371 (http 4, 2026).

Prior to sampling, all equipment, including augers, buckets, sample bags, and labels, was thoroughly cleaned to prevent any cross-contamination between samples. Each of the 12 plots was marked in the field to ensure systematic and consistent sample collection.

A random zigzag sampling pattern was used in each plot to capture spatial variability. The soil sampling depth depended on the root zone of each crop: potato, pea, or lettuce. This ensured the samples reflected the soil environment that influences plant growth. Multiple subsamples were taken from each plot, pooled, and mixed in clean buckets to create representative composite samples.

Each composite sample was then split into subsamples of 500 to 1000 grams. These subsamples were placed in labelled, sealed zip-lock bags to keep them intact during transport. They were quickly delivered to the laboratory for analysis. Detailed records were kept throughout the sampling process to ensure all samples could be traced.

Laboratory analyses were conducted following standardized methods, examining a range of soil parameters as summarized in *Table 3* to evaluate the impact of different treatments on soil quality and fertility.

Table 3: Methods of analysis for soil samples

Lab. Registration Number	Test Parameter	Test Method	Measurement Uncertainty (±R%)
After potato: 232270-232281 After peas: 241,959-241,970 After lettuce: 254207-254218	pH(H_2O), pH(KCl)*	MSZ-08-0206-2:1978 2.1. section	±0.2 pH
	Soil plasticity according to Arany	MSZ-08-0205:1978 5.1. section	± 3 K_A
	Organic matter	MSZ-08-0452:1980 2.2. section, 2.3. section, 3.1.2. section	10
	$CaCO_3$	MSZ-08-0206-2:1978 2.2. section	10
	All water-soluble salts	MSZ-08-0206-2:1978 2.4. section	10
	P_2O_5 (AL)	MSZ 20135:1999 5.1. section	10
	K_2O (AL)	MSZ 20135:1999 5.1. section	10
	$(NO_2^- + NO_3^-)$ - N (KCl)	MSZ 20135:1999 5.4.3. section	10
	Na (AL)	MSZ 20135:1999 5.1. section	10
	Cu (EDTA)	MSZ 20135:1999 5.1. section	10
	Mn (EDTA)	MSZ 20135:1999 5.1. section	10
	Zn (EDTA)	MSZ 20135:1999 5.1. section	10
	Mg (KCl)	MSZ 20135:1999 5.1. section	10
	SO_4^{2-} (KCl)	MSZ 20135:1999 5.1. section	10

Source: Provided by the university laboratory

*In this study, soil pH was determined using a potassium chloride (KCl) solution and H_2O solution. Measuring pH in KCl, rather than in water, provides a more stable and accurate reflection of the soil's active acidity (Food and Agriculture Organization of the United Nations, 2021). The KCl solution displaces exchangeable hydrogen (H^+) and aluminum (Al^{3+}) ions from soil colloids, revealing the potential acidity that may not be apparent in a simple water-based pH test. As a result, pH(KCl) measurements typically yield slightly lower values than pH(H_2O), offering a more reliable indicator of long-term soil acid-base conditions and nutrient availability (Food and Agriculture Organization of the United Nations, 2021).

2.2.5. Growth Measurements and Post-Harvest Sampling Procedures for Potato, Pea, and Lettuce

During the growth phases of the crops, systematic field measurements were conducted to monitor key agronomic parameters across the 12 experimental plots.

Potato: Throughout the potato growth period, measurements were performed directly in the field to assess critical indicators such as the number of plants per plot, average plant height, and weed abundance following the initial weeding approximately two weeks after planting. At harvest, the total potato yield per plot and the average size of harvested tubers were recorded. Sampling after harvest followed a structured zigzag pattern across each plot to ensure representative collection. Potato samples weighing between 500 and 1000 grams were collected into labelled zip-lock bags, with minor variations in sample weight depending on plot yield. Samples were securely sealed and transported to the laboratory for detailed chemical and physical analyses, as specified in *Table 4.*

Table 4: Methods of analysis for potato samples (I year-2023)

Lab. Registration Number	Test Parameter	Test Method
232282-232293	Moisture content	MSZ ISO 1442:2000
	Protein content	MSZ EN ISO 5983-2:2009
	Starch content	152/2009/EK III/L
	Vitamin C content	SZDVL_MU_19

Source: Provided by the university laboratory

Pea: During the pea growth phase, field assessments included measurements of average plant height, average pod length, and the average number of seeds per pod, along with total fresh harvest weight (including pods) per plot. Following harvest, samples were collected using a randomized sampling approach to capture plot variability. Approximately 250 to 300 grams of fresh pea material was gathered per plot into labelled zip-lock bags. These samples were then transported to the laboratory for comprehensive analysis in accordance with the parameters listed in *Table 5.*

Table 5: Methods of analysis for peas samples (II year-2024)

Lab. Registration Number	Test Parameter	Test Method
241,971-241,982	Moisture content	MSZ ISO 6496:2001
	Crude protein content	MSZ EN ISO 5983-2:2009
	Potassium content	MSZ EN ISO 6869:2001
	Phosphorus content	MSZ ISO 6491:2001
	Starch content	152/2009/EK III/L
	Total sugar content	MSZ 6830-26:1987
	Chlorophyll content	AVL_MU N05

Source: Provided by the university laboratory

Lettuce: For lettuce, given the relatively smaller harvest volumes, approximately 300 grams of fresh material was collected per plot from the four treatment groups. Sampling was carefully labelled to correspond with each treatment. All lettuce samples were sealed and transferred to the laboratory for further analysis, where they underwent thorough analysis based on the criteria specified in *Table 6.*

Table 6: Methods of analysis for lettuce samples (III year-2025)

Lab. Registration Number	Test Parameter	Test Method
254219-254222	Moisture content	MSZ ISO 6496:2001
	Phosphorus content	MSZ-08-1783-28:1985
	Calcium content	MSZ-08-1783-26:1985
	Potassium content	MSZ-08-1783-29:1985
	Magnesium content	MSZ-08-1783-27:1985
	Iron content	MSZ-08-1783-31:1985

Source: Provided by the university laboratory

This standardized approach to both field measurement and post-harvest sampling ensured accuracy, reproducibility, and integrity of the data collected across all crops and treatment plots.

2.2.6. Soil Physical Properties Assessment: Penetration Resistance, Moisture Content, and Infiltration Rate

To evaluate the physical condition of the soil under different tillage systems and microbial inoculation treatments, a series of field and laboratory assessments were conducted.

This part of the study focused on three key soil physical parameters: soil penetration resistance, soil moisture content, and soil infiltration rate, which collectively provide insights into soil compaction, water availability, and hydrological performance. These parameters were measured across 12 experimental plots during the cultivation of peas (in the middle of the experiment) enabling comparative analysis across crop types and treatment combinations.

Soil Penetration Resistance

Soil penetration resistance was assessed as an indicator of soil compaction and its potential influence on root development, water movement, and microbial activity. Higher resistance values typically signify greater compaction, which may inhibit root penetration and reduce crop productivity.

- **Instrumentation and Crop-Specific Methods:**
  - For pea cultivation, a penetrometer calibrated in kilopascals (kPa) was utilized. This device, provided by the university, allowed for precise pressure readings at depth (shown in *Figure 10/1).*

    - For lettuce cultivation, a penetrometer with a color-coded scale (green: low resistance, yellow: moderate, red: high resistance) was used (shown in *Figure 10/4*).

- Depth Intervals and Replication: Measurements were taken at four soil depth intervals: 0-10 cm, 10-20 cm, 20-30 cm, and 30-40 cm. Each depth was assessed three times per plot at randomly selected points to ensure statistical reliability.

- **Timing of Measurements:**

    - For peas, measurements were taken at three growth stages: before planting, during early vegetative growth, and after harvest.

    - For lettuce, measurements were conducted before planting and post-harvest.

These measurements allowed for the analysis of soil compaction dynamics over time and under different treatment regimes.

Soil Moisture Content

Soil moisture content was evaluated to determine the availability of water in the root zone, which is essential for plant uptake, nutrient transport, and microbial activity.

- **Sampling Procedure:**

  For soil moisture analysis, samples were collected from four depth intervals (0-10 cm, 10-20 cm, 20-30 cm, and 30-40 cm) in each plot, using soil auger (*Figure 10/2*). The soil from each depth was placed in individual zip-lock bags to preserve its moisture content during transport. Each plot yielded four separate samples, one per depth, which were immediately weighed in the field to record the fresh (wet) mass, then transported to the laboratory for further processing.

- **Timing:**
  One sampling session was conducted during the active growth phase of each crop: once during pea cultivation and once during lettuce cultivation.

- **Laboratory Analysis:**

  In the lab, each sample was again weighed for its fresh (wet) mass, then placed in a drying oven at 105°C for 24 to 48 hours, or until a constant dry weight was achieved. After drying, the samples were reweighed, and gravimetric soil moisture content was calculated using the formula:

$$\text{Soil Moisture (\%)} = \frac{\text{Wet Weight} - \text{Dry Weight}}{\text{Dry Weight}} \times 100$$

Soil Infiltration Rate

Soil infiltration rate is a key indicator of soil structure and porosity, affecting water availability, erosion risk, and nutrient leaching. Field infiltration tests were conducted using the ring infiltrometer method.

- **Equipment and Procedure:**
  A metal ring infiltrometer (shown in *Figure 10/3*) was inserted vertically into the soil to a uniform depth across all plots. A measured volume of water was poured into the ring using a calibrated measuring cup.
- **Observation Intervals:**
  The infiltration of water into the soil was timed and recorded at 1, 2, 3, 4, 5, 10, 15, 20, 25, and 30-minute intervals. The volume of water absorbed at each interval was recorded to construct infiltration curves, representing the dynamics of water movement in the soil profile.
- **Repetitions:**
  Each measurement was conducted once per plot, before and after planting, and data were used to evaluate differences in infiltration behaviour between treatment groups.

Figure 10: Soil Penetrometer (calibrated in kPa) 1., Soil Auger for Moisture Sampling 2., Infiltration Ring 3., and Soil Penetrometer (color-coded scale) 4.

Source: Own work

To evaluate all the results, several methods were used. Graphs were generated in R and merged for each analysis to provide a clear visual representation. Mean data tables were compiled to summarize the central tendencies across groups and time points. ANOVA tables were included to assess the statistical significance of group differences. Additionally, pairwise comparisons using the Tukey test were conducted for each analysis and time point to further explore specific group differences. All results are presented in detail in the Results section.

Due to time constraints and capacity limitations, the aim is to provide a general overview of soil penetration, moisture, and infiltration during the experiment. Therefore, the Results and Discussion section focuses solely on the measurements taken during the pea growth period, which was the II year of the experiment (middle year).

2.2.7. Comprehensive Shotgun Metagenomic Sequencing of Soil Microbiomes: Methodology and Analytical Approach

To determine the microbial community composition and potential functional capabilities in a given soil sample, for this research, soil samples were sent to Xenovea Szolgáltató Kft. to use their paid services to determine and perform metagenomic sequencing. This approach allows comprehensive analysis of all DNA present in the sample, not just targeted genes (e.g., 16S rRNA).

Because shotgun metagenomic sequencing is expensive, the soil samples (three from the same field) have been sent to the sequencing facility in Szeged only once, at the start of the research. This first sequencing aimed to give a general look at the microbial community structure and functional potential of the soil studied here. The goal was to create baseline data that could guide the overall context of the study, not to support detailed long-term or comparative analysis.

Shotgun Metagenomic Sequencing Methodology

Sample Collection and DNA Extraction

- Soil Sampling: 50-100 mg of soil was used for each analysis.
- Three soil samples were randomly collected from the garden, taken from the top 40 cm of soil. Each sample was placed in a separate zip bag. The bags were transported to the laboratory the following day in insulated cool bags to maintain optimal temperatures for microbial preservation.
- DNA Isolation: Total metagenomic DNA was extracted using the ZymoBIOMICS 96 MagBead DNA Kit following the manufacturer's protocol (Zymo Research). This method ensures the isolation of high-quality DNA suitable for downstream sequencing.

Library Preparation and Sequencing

- The purified DNA was processed for shotgun metagenomic sequencing, where the entire DNA content of the sample is randomly fragmented and sequenced.
- Sequencing was performed using the NovaSeq platform (Illumina), generating >20 million reads per sample, enabling high-resolution profiling of the microbial community.

Data Preprocessing

- Demultiplexing and Adapter Trimming: Sequencing reads were demultiplexed and trimmed to remove adapter sequences using NovaSeq Control Software.
- Quality Filtering: Using FastQ Toolkit (Illumina):
    - Bases with a quality score <30 at both 3’ and 5’ ends were trimmed.
    - Reads with:
        - Mean quality score <30, Length <100 bp were discarded to ensure high-confidence data.

Taxonomic and Functional Profiling

- **Taxonomic Classification:**
    - Performed using the DRAGEN Metagenomics Pipeline (v3.5.13, Illumina).
    - This tool maps sequencing reads against a comprehensive microbial reference database to identify microbial taxa.
- **Limitations:**
    - 60-90% of reads could not be uniquely assigned to a reference genome due to:
        - Shared functional genes among microbes (e.g., catabolic and metabolic genes).
        - Absence of taxonomically informative regions.
    - These unclassified reads still contribute significantly to functional potential analyses.
    - Rationale for Conducting Metagenome Sequencing Only Once During Field Trial:
        - Metagenome sequencing was performed only once in this study, before planting the first plant. The primary objective was to obtain a comprehensive overview of the garden’s existing microbial community structure and to use this baseline to guide future decisions regarding microbial application. Establishing this initial profile enabled more informed recommendations on which microbial strains would be most appropriate for application.
        - Additionally, the study aimed to reflect realistic conditions encountered in practical horticulture and agriculture. In real-field settings, farmers and gardeners rarely conduct annual metagenomic analyses due to the high cost and limited accessibility of such methods. By restricting sequencing to a single assessment, the study aimed to emulate these constraints and ensure that the research framework remained applicable to common practice.
        - While annual sequencing would provide more frequent insights into microbial dynamics, it would not be economically feasible or representative

of standard field management. Instead, it may be more realistic and valuable to conduct follow-up metagenome sequencing at longer intervals, such as after approximately five years, to evaluate whether the introduced microbial interventions have led to measurable improvements in soil microbial composition.

Data Interpretation and Applications

- Microbial Composition:
    - Reliable microbial distribution is derived from reads uniquely mapping to conserved genomic regions.
    - Visualized with interactive tools like Krona charts for detailed exploration.
- Functional Insights:
    - Reads not mappable to taxa are analyzed using deep bioinformatic tools to infer:
        - Catabolic pathways
        - Metabolic routes
    - This allows insights into microbial functions in the soil ecosystem, such as nutrient cycling or pollutant degradation.

Data Storage Policy

- In compliance with internal and MATE regulations, raw sequencing data are stored for one year only. After that, the datasets are deleted as per policy.

Shotgun metagenomic sequencing is a powerful, untargeted approach for analysing the taxonomic diversity and functional capacity of soil microbiomes. While some sequencing reads remain taxonomically unassigned, they provide valuable insights into the metabolic capabilities of the microbial community through advanced bioinformatics. The results of this part are presented in the Results section.

2.2.8. NDVI Measurement and Its Relevance in Pea Cultivation

During the pea growing period, as the crop developed into a dense green canopy, the Normalized Difference Vegetation Index (NDVI) was measured in collaboration with colleagues from the HUN-REN-MATE Agroecology Research Group, Department of Plant Physiology and Plant Ecology, Institute of Crop Production Sciences.

NDVI is a key remote sensing indicator that provides insights into plant vitality, chlorophyll content, and photosynthetic activity, making it an extremely valuable tool for monitoring crop growth and overall health (Kurouski, 2021).

Its strength lies in offering an objective, spatially detailed assessment of vegetation conditions, allowing researchers to detect subtle differences in plant performance across the field that are difficult to observe with the naked eye.

In this research, drone-based imaging was applied to collect high-resolution data, from which NDVI maps were produced and later presented in the Results section (Results for Physical Properties of Potato, Peas, and Lettuce).

NDVI measurements were conducted only during the pea growth period because peas develop a distinctly larger and greener canopy compared to potato and lettuce. This makes them particularly suitable for detecting differences in vegetation performance using NDVI. In the study by Marzougui et al. (2023), peas were grown from entries that produced either green or yellow seeds, and all seeds were inoculated with *Rhizobium* bacteria prior to sowing to ensure effective nitrogen fixation. NDVI was then used as a non-destructive tool to assess plant health, estimate yield potential, and evaluate overall crop quality.

The obtained values ranged from 1 to -0.85, where positive values correspond to vegetation cover, while negative or near-zero values typically represent bare soil, dead plant material, or other non-vegetative surfaces.

For practical interpretation, the following classification scale was used:

- values from -1 to 0 represent dead plants, bare soil, or non-plant objects;
- 0 to 0.33 characterize unhealthy plants;
- 0.33 to 0.66 represent moderately healthy plants;
- 0.66 to 1 are indicative of healthy, vigorous vegetation.

Getting NDVI measurements is especially helpful. It enables a non-invasive, unbiased, and consistent evaluation of plant health changes over time. This supports both farming decisions and scientific understanding of crop performance in the specific experimental conditions.

2.2.9. Statistical Evaluation: Analysis Using Box Plots and ANOVA in R

To evaluate the effects of different treatments, there was a need to conduct statistical analyses using the R programming environment. It has been used a one-way Analysis of Variance (ANOVA) to see if there were significant differences in mean values among the treatment groups. This method works well for comparing multiple groups, like no-tillage and loosening techniques, with and without microbial applications.

Box plots were created to visually show the distribution of data within each treatment group. These plots displayed central trends, variability, and any potential outliers. The ANOVA was performed under the assumption that there were no significant differences among the group means. The significance level at $p < 0.05$ has been set to determine statistical relevance.

When ANOVA showed statistically significant differences ($p < 0.05$), there was a need to conduct post-hoc analysis to find out which specific group means differed. Tukey Honest Significant Difference (Tukey HSD) test on the ASTATSA online platform was used. The resulting p-values and F-statistics confirmed that at least one pair of treatment groups showed a significant difference, which supported further pairwise comparisons.

3. RESULTS AND DISCUSSION

This Results section presents a detailed analysis of the physical and chemical properties of the crops and soil, as well as the microbial composition of the soil.

It begins with the physical properties of the potato (2023) and peas (2024), which were measured in the field. This section also includes NDVI map created during peas growth. These measurements include growth parameters such as size, weight, and yield, which were tracked over the course of the growing season. In the case of lettuce, due to the limited quantity obtained at harvest, sufficient only for laboratory analysis, no direct measurements of physical properties (e.g., head weight, size, or firmness at harvest) were carried out. Additionally, due to limitations in time management and the necessity of transporting the samples to the laboratory. Nevertheless, the visual evaluation of the harvested produce revealed that the lettuce heads consistently exhibited a uniform and vibrant green coloration, which is generally considered an indicator of good physiological condition and high marketable quality.

The chemical properties of the potato (2023), peas (2024), lettuce (2025), and soil after each planting season, were also analysed in the laboratory after each year of the study. These analyses provide insight into the nutrient composition, moisture content, and other relevant factors that influence plant health and soil fertility.

The section further discusses the results from soil penetration, soil moisture, and soil infiltration tests, which were measured during the cultivation of peas (2024). These measurements help to evaluate the structural properties of the soil, including its ability to retain moisture and support plant growth, as well as how quickly water infiltrates the soil.

Finally, the section includes results from shotgun metagenomic sequencing of the soil microbiomes. This advanced sequencing technique provides a detailed understanding of the microbial communities present in the soil, offering insights into their diversity, composition, and potential roles in nutrient cycling and overall soil health.

Together, these findings offer a comprehensive view of the factors that contribute to soil quality, crop growth, and the ecological interactions between plants and soil microorganisms.

3.1. Physical Properties of Potato, Peas, and Lettuce

Table 7 presents detailed measurements from four distinct plot treatments: A- (loosening without microbes), B- (no-tillage without microbes), A+ (loosening with microbes), and B+ (no-tillage with microbes). The parameters of potato evaluated include averages of the number of plants per plot, average plant height, average weed abundance, average harvest yield, and the average dimensions of potato plants.

Table 7: Summary of the physical properties of potato (I year-2023)

Treatment	**Average Number of Plants in Each Plot**	**Average Height cm**	**Average Amount of Weeds g**	**Average Harvest g**	**Average Dimension cm^2**
A-	4	32	1667	755	41.33
B-	5	33	2067	842	48.50
A+	3	32	733	685	39.00
B+	3	28	867	615	30.50

A-: Loosening (No microbes); B-: No-tillage (No microbes); A+: Loosening with Microbes; B+: No-tillage with Microbes

Source: Own work

Each plot was planted with seven potato plants. **As presented in Box plots in *Figure 11*, none of the measured parameters for potato showed statistically significant differences, the observed presented data suggests a pattern, used to facilitate interpretation and contextual understanding of the results.**

Regarding **number of plants in each plot of potato (*Figure 11/A*)**, the B- (no-tillage no microbes) treatment group showed the greatest average count, with five plants recorded to grow successfully. Plots lacking microbial application tended to have higher plant counts compared to those treated with microbes. For example, in the A+ (loosening with microbes) treatment group, the average dropped to three plants. This may suggest that the introduction of microbes, when paired with soil management strategies, may reduce plant establishment, possibly due to the intricate relationships between microbial activity and plant development. As emphasized in the study by Mukhametov et al. (2023), potato cultivation can be managed through diverse technological approaches, each leading to different productivity outcomes. While the experiment's consistent planting of seven seed potato per plot allowed for reliable comparison, exploring additional factors such as irrigation during sprouting or the quality of seed material could provide deeper insights into refining potato production methods.

When considering **plant height of potato (*Figure 11/B*)**, the B- (no-tillage no microbes) treatment group recorded the tallest plants on average. A comparison between plots treated with microbes and those without shows that microbial application did not markedly influence plant height across treatments. This points to the likelihood that variables such as soil structure, nutrient supply, or environmental factors play a more decisive role in determining plant growth than microbial presence alone. Reference to existing standards indicates that the average heights observed across all treatments are consistent with the expected growth range for potato plants, typically between 45 cm and 90 cm at maturity (http 5, 2026). This alignment suggests that, although cultivation methods and microbial treatments may exert some influence on growth, the plants still follow the characteristic developmental patterns typical of their species.

Regarding the **average amount of weeds in potato planting (*Figure 11/C*)**, the highest values were again observed in the B- (no-tillage no microbes) treatment group, indicating that the absence of tillage strongly contributed to increased weed proliferation. In contrast, plots under A+ (loosening with microbes) and B+ (no-tillage with microbes) treatments consistently exhibited lower weed abundance than those in A- (loosening without microbes) and B-, suggesting that the combined application of microbial inoculants and soil loosening may play a suppressive role. This combination appears to function in a manner comparable to herbicidal activity, offering potential as a strategy for more effective weed control. Nevertheless, additional investigation is required to disentangle the individual contributions of microbial presence, soil disturbance, and no-tillage practices to overall weed dynamics. As emphasized by Horvath et al. (2023), weeds exert their most significant impact early in the growing season, when nutrient availability from fertilizers and soil moisture is high. This underscores the importance of early intervention in weed management. Their findings also suggest that the detrimental effect of weeds may extend beyond direct competition for resources, particularly in well managed agricultural systems. Taken together, the present results highlight the multifactorial nature of weed management. While the integration of microbes with soil manipulation practices shows promise for suppressing weed populations, further research is essential to clarify the mechanisms driving these outcomes and to refine integrated strategies for maximizing crop productivity.

When considering the average **harvest of potato (*Figure 11/D*)**, the B- (no-tillage no microbes) treatment tended to have the highest average recording 842 g, followed by A- (loosening without microbes) with 755 g, A+ (loosening with microbes) with 685 g, and B+ (no-tillage with microbes) with 615 g. This distribution shows that while the absence of tillage B- supported higher yields, the combination of microbes with tillage. B+ resulted in the lowest harvest among the treatments. These results suggest that the incorporation of microbes alongside soil management practices, such as loosening, may enhance potato yield. Nevertheless, further investigation is required to clarify the mechanisms responsible for the observed differences in harvest performance.

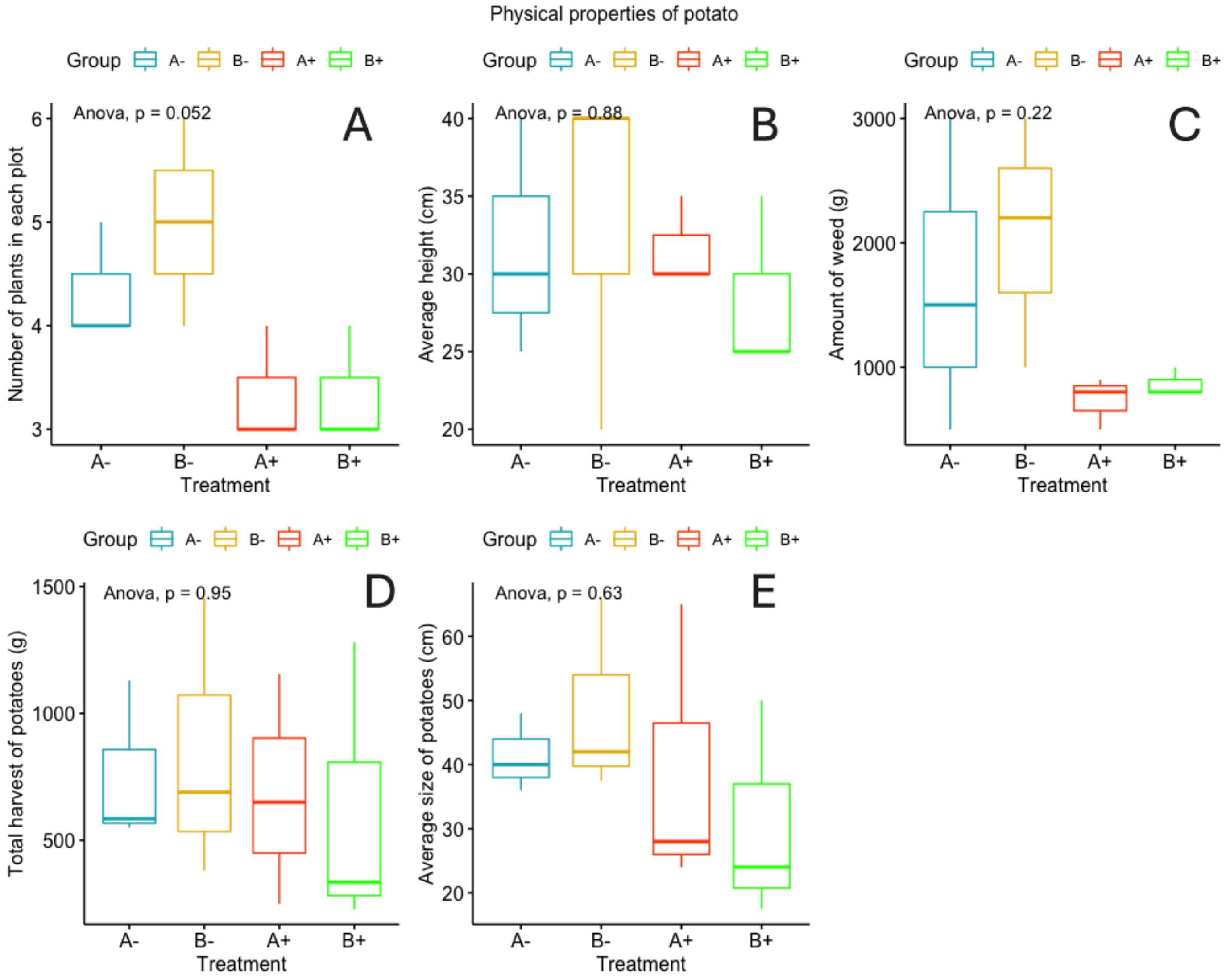
The average size of potato tubers (*Figure 11/E*) is influenced by multiple factors, including varietal characteristics, environmental conditions, and agricultural practices such as cultivation techniques, fertilization regimes, and pest control strategies. According to established agricultural standards, the average dimensions of a potato typically range between 5-10 cm in length and 4-6 cm in width (http 5, 2026). These values may vary slightly depending on the potato variety and regional growing conditions. In the present study, the anticipated average potato size was consistent with this general range, providing an appropriate reference point for interpreting size variations among treatments. As reported by Saini et al. (2021), the potato tubers treated with microbial inoculants often achieve larger sizes and greater biomass compared to untreated plots, largely due to enhanced nutrient uptake efficiency.

Overall, after the first year of applying microbial inoculation, the outcomes across all experimental plots aligned closely with expectations reported in the literature regarding plant establishment and survival. Most potato plants developed successfully, displaying growth and a healthy appearance, while the tubers harvested were of satisfactory size and quality. These findings indicate that the cultivation system was stable and capable of supporting productive growth under the tested conditions.

Although statistical analysis did not reveal significant differences between the treatments, certain consistent numerical differences were observed. Microbial inoculation appeared to have only a modest influence on plant performance. Importantly, no negative interactions were observed, suggesting that the addition of microbial inoculants can be integrated into production systems without compromising plant health or yield. This neutral-to-positive effect is noteworthy, as it indicates the compatibility of microbial treatments with conventional cultivation practices. The absence of pronounced differences also underscores the complexity of plant-microbe-soil interactions, where multiple factors such as soil properties, nutrient availability, climatic conditions, and tillage practices may play more dominant roles than microbial inoculation alone. Nevertheless, the observed patterns provide a useful basis for future research aimed at disentangling these interactions. **In summary, microbial inoculation during the first year did not significantly alter yield outcomes but indicated compatibility with existing cultivation methods, offering potential as a sustainable practice that warrants further investigation.**

Figure 11 includes a summary of the physical properties of potato in form of Box Plots created in R, along with corresponding p-values to highlight statistical differences.

Figure 11: Summary of the physical properties of potato with p-values (I year-2023)



** p-value > 0.05-no significant differences between treatments; p-value < 0.05-significant differences between treatments*

A-: Loosening (No microbes); B-: No-tillage (No microbes); A+: Loosening with Microbes; B+: No-tillage with Microbes

Source: Own work

Table 8 provides an overview of measurements from the same four plot treatments: A-, B-, A+, and B+. The parameters of peas assessed include average number of seeds per pod, average plant height, average pod length, and the total harvest weight (including pods) in grams.

Table 8: Summary of the physical properties of peas (II year-2024)

Treatment	Average Number of Seeds in the Pod	Average Height in cm	Average Pod Length in cm	Total Harvest in g (with Pods)
A-	7	58.93	6.10	2343
B-	8	56.93	5.83	2517
A+	8	59.47	6.07	2185
B+	8	57.07	5.80	2235

A-: Loosening (No microbes); B-: No-tillage (No microbes); A+: Loosening with Microbes; B+: No-tillage with Microbes

Source: Own work

Analysing physical properties of peas in *Figure 12*, **none of the measured parameters for peas demonstrated statistically significant differences. The observed, presented data suggests a pattern, used to facilitate interpretation and contextual understanding of the results.**

Looking at **the average number of seeds per pod in peas (*Figure 12/A*)**, the A+ (loosening with microbes) plots produced higher seed counts on average, with replications averaging eight seeds per pod. By comparison, the B+ (no-tillage with microbes) treatment showed more variable outcomes, averaging around seven seeds per pod, suggesting that microbial inoculation is most effective when combined with soil loosening to support reproductive development. According to Dawa et al. (2014), the optimal range for pea pod seed counts is typically 6 to 8, although certain varieties may produce up to 9 or 10 seeds per pod. Their findings further suggest that the application of biofertilizers directly influences this trait, with the dual inoculation treatment (seeds and soil) achieving the highest seed numbers, followed by seed-only inoculation, while the uninoculated A- (loosening without microbes) produced the fewest seeds.

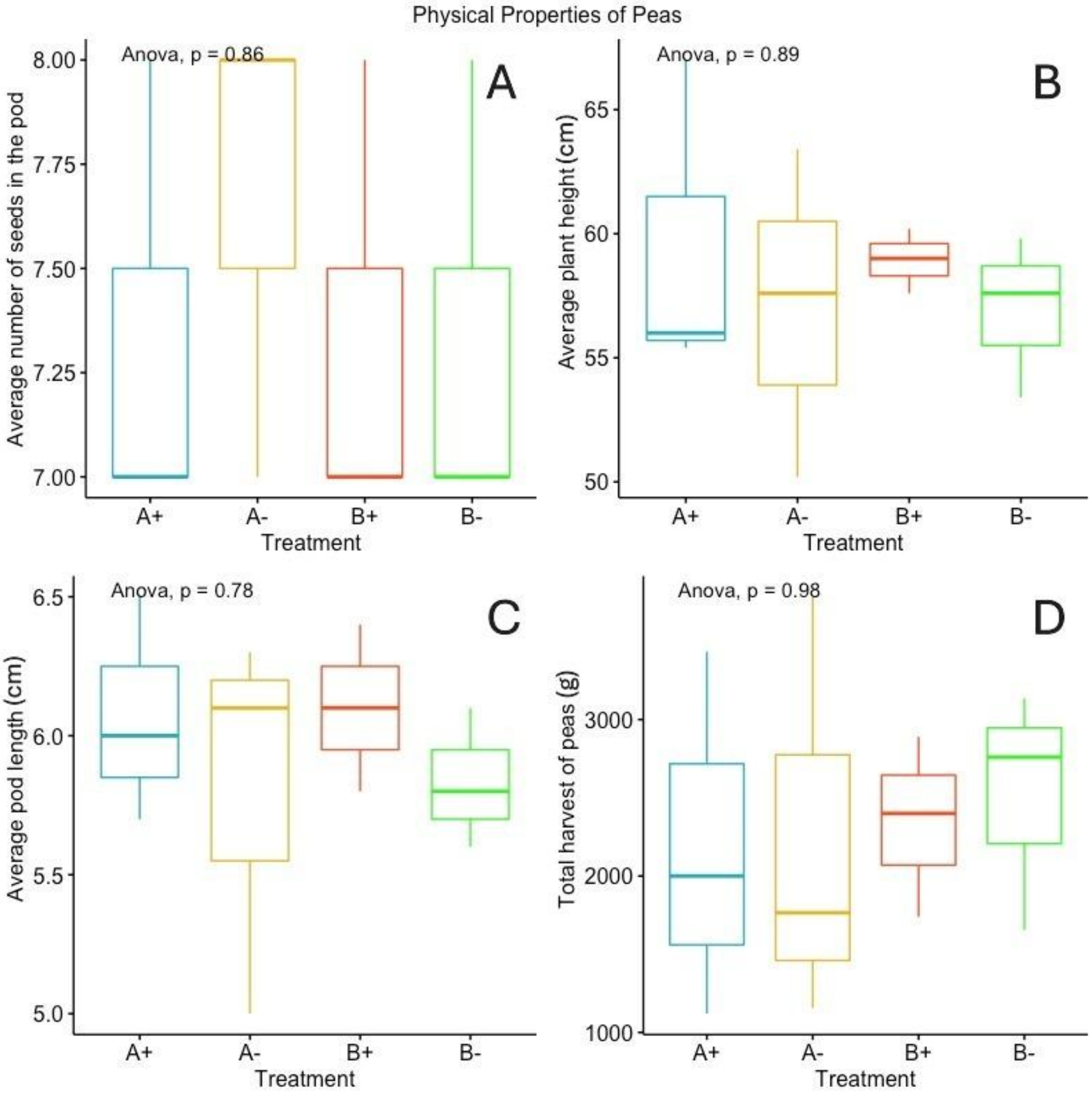
The results indicate that soil loosening treatments (A+ and A-) produced greater **average pea heights (*Figure 12/B*)** compared to the no-tillage treatments (B+ and B-). Among these, the A+ (loosening with microbes) plots recorded the tallest plants, demonstrating that loosening the soil, particularly in combination with microbial inoculation, provides more favorable conditions for pea growth than no-tillage approaches. This outcome suggests the synergistic effect of soil loosening and microbial inoculants on crop performance. By improving aeration and water penetration, soil loosening creates an environment that supports microbial activity and root development. At the same time, inoculation introduces beneficial microorganisms that can establish more effectively in loosened soil, where they enhance nutrient cycling, accelerate organic matter breakdown, and stimulate plant root systems. Together, these processes contribute to better soil structure, greater nutrient availability, and improved overall plant growth. Numerous studies have proven the positive influence of plant-growth-promoting rhizobacteria (PGPR) on crop development. For instance, Ejaz et al. (2020), reported significant improvements in pea plant height following inoculation with beneficial microbial strains. Similarly, the work of Pattnaik et al. (2019), reveals that direct plant growth promotion is strongly influenced by microbial species such as *Rhizobium* spp., *Pseudomonas* spp., and *Bacillus* spp., which contribute to nutrient availability, hormone regulation, and root development.

With respect to **pod length in peas (*Figure 12/C*)**, the A (loosening) treatments once again outperformed the B (no-tillage) categories. This pattern presents the numerical difference without significance and the positive influence of soil loosening combined with microbial inoculation on pod development, likely through the promotion of stronger root systems capable of improved nutrient and water acquisition. Pod length in peas is closely associated with the efficiency of nutrient uptake (notably nitrogen, phosphorus, and potassium), as well as water-use mechanisms. Enhanced nutrient acquisition typically increases pod size, while improved water retention, facilitated by deeper root growth and better stomatal regulation; helps sustain pod development even under water-limited conditions. Evidence from Dawa et al. (2014), further supports this, showing that biofertilizer application significantly enhances pod length. The greatest effect was observed with dual inoculation (seeds and soil), followed by seed-only inoculation, while the uninoculated plots consistently produced the shortest pods.

The total harvest weights of peas (*Figure 12/D*) closely reflected the observed patterns in plant height, pod length, and seed count. B- (no-tillage no microbes) showed the highest average of total harvest, followed by A- (loosening without microbes) treatment. When comparing treatment groups, the A plots (loosening) yielded higher averages than the B plots (no-tillage), reinforcing the importance of soil disturbance and microbial activity in maximizing pea productivity. This outcome underscores the beneficial role of combining soil loosening with microbial inoculation in promoting crop productivity. The study by Dawa et al. (2014), presented that biofertilizer use can substantially increase pea yields, particularly under dual inoculation (seeds and soil). Similarly, Mahmoud et al. (2023), reported that microbial inoculation, especially with *Azotobacter* and *Bacillus* species, enhances both crop performance and soil health. Such inoculants improve nutrient uptake and plant metabolism, raising chlorophyll levels, stimulating carbohydrate pathways, and promoting phytohormone production, all of which ultimately support higher biomass and yields.

Figure 12 includes a summary of the physical properties of peas in form of Box Plots created in R, along with corresponding p-values to highlight statistical differences.

Figure 12: Summary of the physical properties of peas with p-values (II year-2024)



** p-value > 0.05-no significant differences between treatments; p-value < 0.05-significant differences between treatments*
A-: Loosening (No microbes); B-: No-tillage (No microbes); A+: Loosening with Microbes; B+: No-tillage with Microbes
Source: Own work

In general, the A+ (loosening with microbes) treatment mostly performed best across measured parameters, suggesting a strong synergistic effect between microbial inoculation and soil loosening. While A- (loosening without microbes) treatments delivered some positive results, they remained below A+ performance, indicating the crucial contribution of beneficial microbes. At the same time, in the broader dataset, the B- (no-tillage no microbes) treatment produced the highest average yield overall. Yet, within other treatments, particularly A+ and A-, there were individual cases that rivaled or surpassed certain B- plots. This variability suggests that while no-tillage without microbial inoculation can generate high yields on average, specific conditions within loosened or inoculated plots may also create favorable outcomes. Such findings highlight the heterogeneity of plant responses, shaped not only by treatment type but also by localized soil properties, microclimatic variations, and seed quality.

Figure 13 presents the harvests of potato, peas, and lettuce, providing a visual representation of their appearance. As observed in *Figure 13* and in the results, the physical characteristics of the crops over the various experimental years, it is evident that the plants consistently displayed a robust, well-formed, and good appearance. The stable growth patterns and distinct coloration suggest that the management practices employed created favorable conditions for the plants. Moreover, as the experiment progressed over multiple years, consistent patterns in plant performance were observed, with numerical differences indicating higher values over time, without significant differences. These observations suggest that the ongoing trial may have been associated with gradual improvements in plant performance, potentially linked to cumulative effects on soil structure, increased microbial activity, or the stabilization of nutrient dynamics within the agroecosystem. These long-term developments underscore the importance of sustained experimental efforts, as short-term evaluations may miss the incremental benefits of ecological management practices on crop health and quality.

Figure 13: Final Harvest from Potato (2023), Peas (2024), and Lettuce (2025) Plants

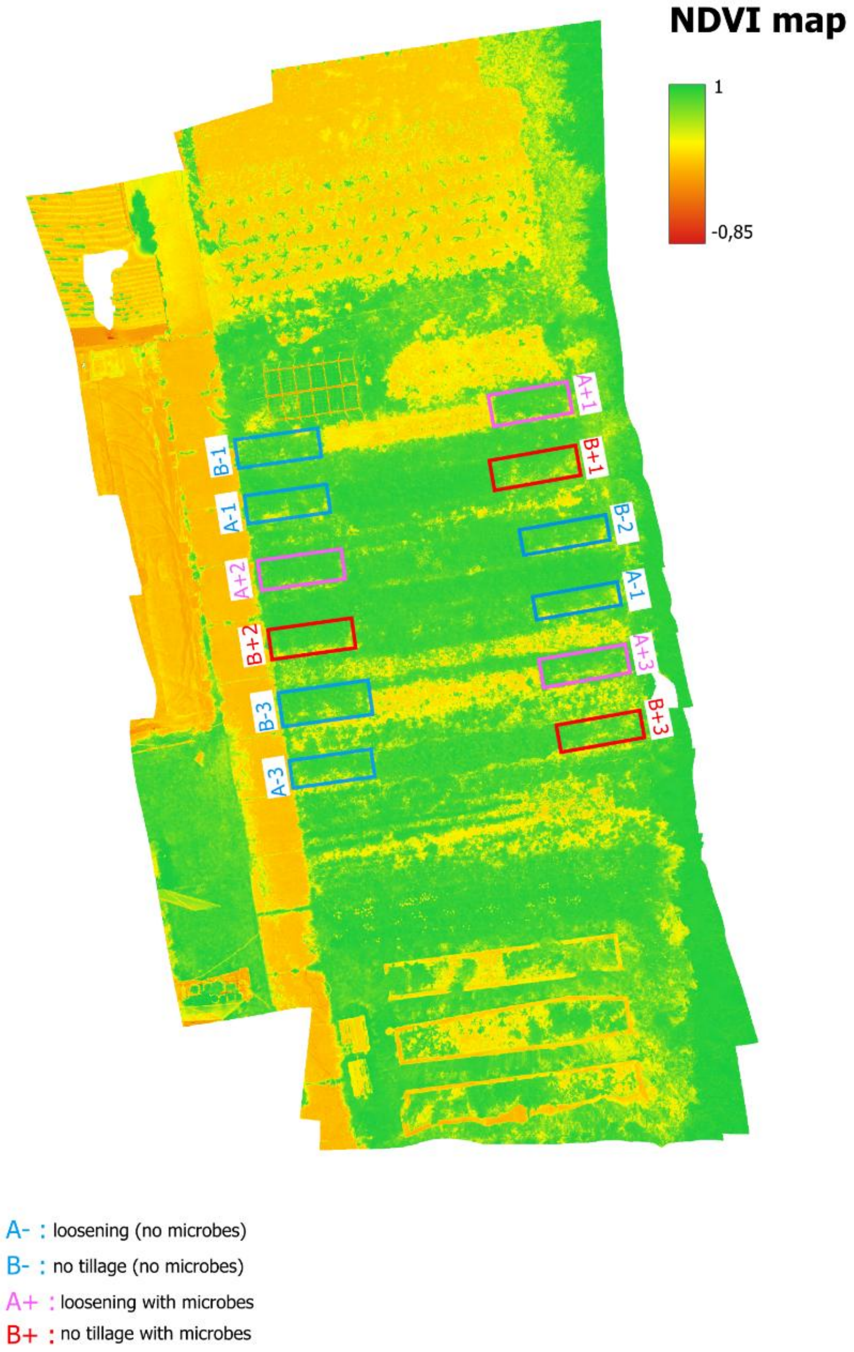
Source: Own work

3.1.1. NDVI Map of Pea Crop Development

Figure 14 shows a detailed NDVI map illustrating pea crop development across four different plot treatments: A- (soil loosening without microbial inoculation), B- (no-tillage without microbial inoculation), A+ (soil loosening with microbial inoculation), and B+ (no-tillage with microbial inoculation). During the growth period of peas, Normalized Difference Vegetation Index (NDVI) measurements were performed to monitor and quantify the vegetation status of the crop plots. The spatial NDVI map, illustrates that the majority of experimental plots consistently fell within the higher NDVI ranges, corresponding with intense green coloration on the map. Such NDVI values are indicative of healthy, actively photosynthesizing vegetation and are widely recognized as proxies for healthy plant development and robust canopy establishment. The observed spatial patterns suggest that the peas cultivated during the trial period exhibited optimal physiological status, with no signs of significant stress or decline typically associated with lower NDVI values.

These findings provide quantitative support to the visual assessments of crop health and further validate the effectiveness of the management practices in promoting strong and healthy vegetation throughout the experimental plots. The integration of NDVI mapping with qualitative observations allows for a comprehensive evaluation of pea growth dynamics and further understanding of the spatial variability in crop performance under the given agroecological conditions.

Figure 14: NDVI Map of Pea Crop Development Under Different Soil Management and Microbial Treatments (II year-2024)



Source: Own work

3.2. Chemical Properties of Potato, Peas, and Lettuce

Table 9 presents a detailed summary of the chemical properties of potato samples subjected to four distinct treatments: A- (soil loosening without microbial inoculation), B- (no-tillage without microbial inoculation), A+ (soil loosening with microbial inoculation), and B+ (no-tillage with microbial inoculation). The parameters evaluated include moisture content, protein content, starch content, and vitamin C content. Each table presents the average values obtained from three replicates for each of the four treatments.

Table 9: Laboratory results for chemical properties of potato (I year-2023)

Treatment	Moisture Content %	Protein Content %	Starch Content %	Vitamin C Content mg/kg
A-	81.20	1.53	13.50	5.72
B-	82.50	1.47	12.47	8.12
A+	82.83	1.50	11.93	11.32
B+	81.47	1.70	13.37	9.56

A-: Loosening (No microbes); B-: No-tillage (No microbes); A+: Loosening with Microbes; B+: No-tillage with Microbes

Source: Own work

None of the chemical properties of potato showed significant differences between the treatments. Instead, numerical differences observed in the data and the general discussion are presented in the following sections. The results provide insight into how microbial treatments and soil management practices influence these parameters as potential patterns and overall, the findings are consistent with existing literature.

Among the examined categories, the highest average **moisture content of potato (*Figure 15/A*)** was observed in categories A+ (loosening with microbes) and B- (no-tillage no microbes). With this, it can be assumed that the presence of beneficial microbes, coupled with appropriate soil management, may positively influence the water-retaining capacity of tubers. Although this result differs from the findings reported by Saini et al. (2021), who observed increased moisture levels following inoculation, the variation could be attributed to differences in soil type or environmental conditions. Nonetheless, the data suggested a pattern that supports the broader understanding that microbial activity can alter water dynamics in soil.

Protein levels of potato (*Figure 15/B*) were found to be relatively consistent across all treatment categories, indicating that the applied microbial inoculants and cultivation techniques showed minimal influence on the protein content of potato. This observation aligns with well-established data indicating that fresh potato generally contain 1-2% protein (http 6, 2026). This reinforced that protein content is a stable trait in tubers regardless of external agronomic interventions.

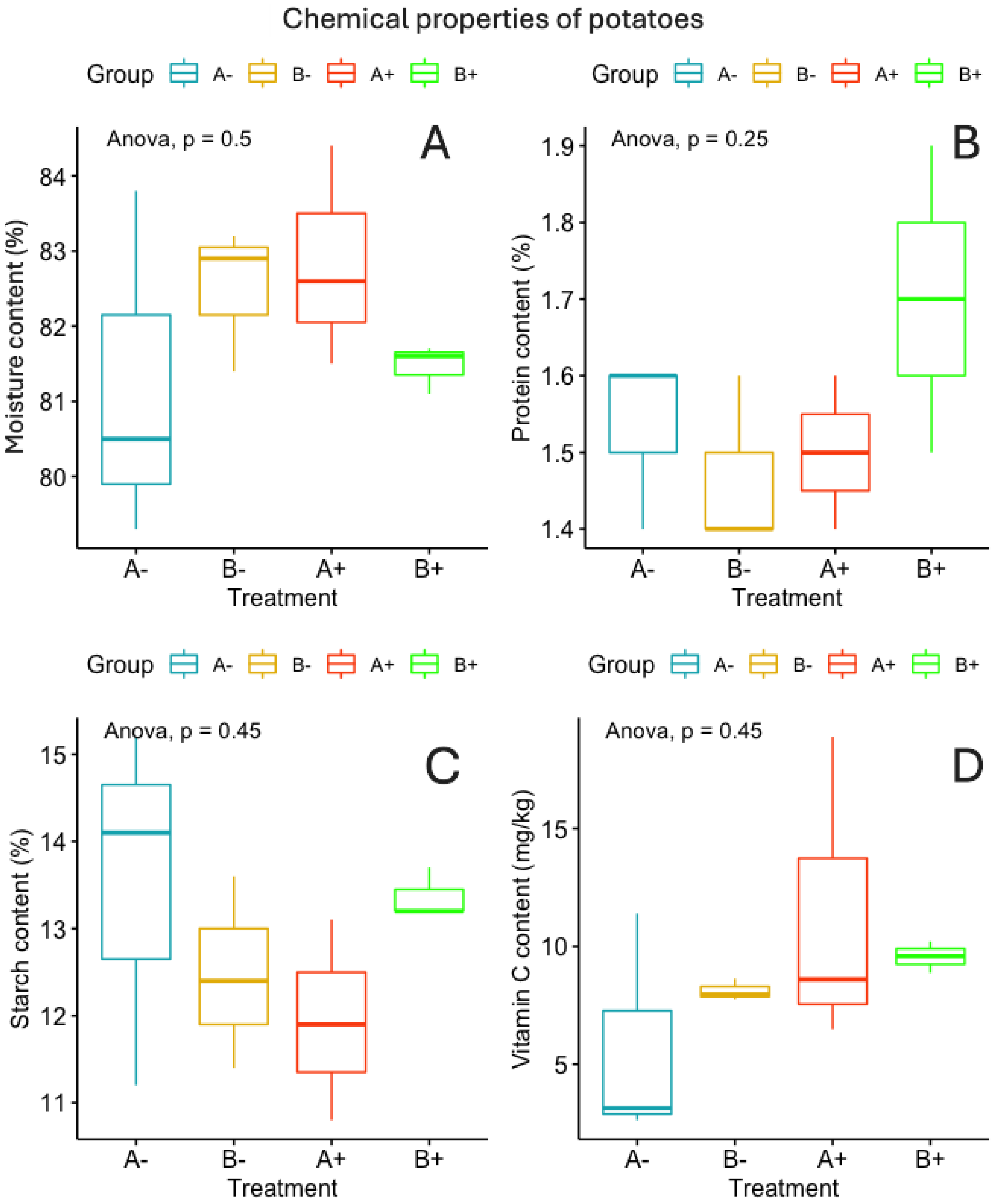
Starch levels of potato (*Figure 15/C*) varied among the different treatment groups, with the highest concentrations found in categories where microbial inoculants were not applied. This result is contrary to the findings of Saini et al. (2021), who reported enhanced starch accumulation following microbial inoculation. However, this discrepancy may be influenced by soil structure and moisture retention capabilities. According to Birkás et al. (2007), sandy loam soils, which are prone to poor water and nutrient retention, can significantly affect crop quality and yield. In such soils, the role of microbial presence may shift, potentially reducing starch accumulation under stress conditions. Despite the divergence from one specific study, the results are still within the expected range reported in the broader literature.

Vitamin C of potato (*Figure 15/D*), a key antioxidant present in potato, showed noticeable differences across treatment categories. The highest average levels were recorded in category A+ (loosening with microbes), indicating that microbial inoculation and optimized cultivation practices may contribute to enhanced vitamin C synthesis. These findings are in agreement with previous studies that link improved soil health and microbial activity to higher vitamin content in crops.

Overall, the chemical composition data suggests a multifaceted interaction between soil management, microbial inoculation, and the nutritional profile of potato. While some findings diverge slightly from individual studies, they remain broadly consistent with scientific literature. The results underscore the importance of integrated cultivation strategies for optimizing both yield and quality. Further investigations are recommended to clarify the mechanistic basis of these interactions and refine agricultural practices for improved tuber chemistry.

Figure 15 presents box plot visualizations summarizing the chemical properties of potato. The figure is based on statistical analyses conducted using one-way ANOVA implemented in the R statistical environment.

Figure 15: Summary of the chemical properties of potato with p-values (I year-2023)



** p-value > 0.05-no significant differences between treatments; p-value < 0.05-significant differences between treatments*
A-: Loosening (No microbes); B-: No-tillage (No microbes); A+: Loosening with Microbes; B+: No-tillage with Microbes
Source: Own work

Table 10 provides a comprehensive overview of the chemical characteristics of pea samples under the same four treatment conditions (A-, B-, A+, and B+). The measured parameters include moisture content, raw protein content, phosphorus content, potassium content, chlorophyll content, starch content, and total sugar content. As with the potato samples, the data represent the mean values from three replicates per treatment.

Table 10: Laboratory results for chemical properties of peas (II year-2024)

Treatment	Moisture Content %	Raw Protein Content %	Phosphorus Content g/kg	Potassium Content g/kg	Chlorophyll Content mg/100 g	Starch Content %	Total Sugar Content %
A-	73.07	8.13	1.53	3.40	20.33	7.47	1.20
B-	72.37	8.43	1.70	3.60	21.30	7.23	1.53
A+	73.20	7.90	1.53	3.33	17.14	7.40	1.17
B+	74.50	7.57	1.50	3.33	19.24	6.80	1.27

A-: Loosening (No microbes); B-: No-tillage (No microbes); A+: Loosening with Microbes; B+: No-tillage with Microbes

Source: Own work

Most of the chemical properties of peas did not show statistically significant differences among treatments. Instead, numerical differences observed in the data and the resulting patterns are discussed in the following sections. Overall, the results provide insight into how microbial treatments and soil management practices may influence these parameters, and the observations are generally consistent with existing literature. Where a statistically significant difference was detected, notably for total sugar content, a more detailed discussion is provided.

Among the various treatments investigated, the highest average **moisture content of peas** (***Figure 16/A***) was present in treatment B+ (no-tillage with microbes), suggesting that microbial activity contributes positively to soil moisture retention, even under minimal soil disturbance. These findings underscore the potential benefits of integrating microbial inoculants into no-tillage systems to enhance water retention capacity and overall plant health. Supporting literature reinforces these observations Ganjloo et al. (2018), assessed several physical properties of pea seeds across a wide range of moisture contents, from 75.15% in freshly harvested seeds to 15.21% after drying. While their findings reflect similar results in moisture variation as in this research, it is important to note that these values are not directly linked to microbial presence. The moisture content across the different treatments in their study remained relatively consistent, indicating that factors other than microbial activity may play a dominant role in seed moisture retention.

In terms of **raw protein content of peas (*Figure 16/B*)**, the results indicate a slight decline across the different treatments. The highest average protein concentration, 8.43%, was observed in the B- treatment (no-tillage no microbes), without significant difference. However, this suggests that the no-tillage approach may promote protein accumulation, potentially due to enhanced nutrient retention and improved root development in undisturbed soils. In contrast, the A treatments, particularly A+ (soil loosening with microbes), exhibited lower protein levels. This observation may imply that the physical disturbance associated with soil loosening could impair nutrient uptake, possibly by disrupting soil structure or microbial communities. These findings align partially with those of Małecka-Jankowiak et al. (2016), who reported that long-term no-tillage systems did not negatively affect protein content in pea grains. Their study shows that protein yield was closely associated with overall grain yield, and not significantly influenced by the type of tillage system employed. This suggests that while tillage may influence nutrient dynamics and soil structure, its direct impact on protein content in pea grains may be limited.

Phosphorus content of peas (*Figure 16/C*) remained relatively stable across all treatments, with most samples showing concentrations around 1.5 g/kg. This consistency may be attributed to the pea plant's inherent ability to efficiently remobilize phosphorus from older tissues to younger, actively growing tissues, particularly during the reproductive phase. Such internal nutrient redistribution can mitigate the effects of external soil management differences. Nonetheless, the no-tillage treatments, most notably B- (no-tillage no microbes), suggested a slight advantage in phosphorus levels, suggesting that reduced soil disturbance may enhance phosphorus retention and availability. These observations are consistent with broader principles of nutrient cycling in agroecosystems, highlighting the critical role of soil management in maintaining nutrient accessibility and supporting plant development.

Potassium content of peas (*Figure 16/D*) exhibited notable variability among the treatments, with the averages highest concentrations recorded in the B- (no-tillage no microbes) treatments, followed by A- (loosening with no microbes) treatments. These elevated levels suggest that microbial inoculation plays role in enhancing potassium availability, likely facilitated by the decomposition of organic matter and the weathering of potassium-bearing minerals. This enhancement can be attributed to microbial activity improving nutrient mobilization in the rhizosphere. Supporting this observation, Quddus et al. (2024), reported that *Rhizobium* inoculants can directly influence plant metabolism by solubilizing phosphates and producing plant growth-promoting hormones, thereby contributing to improved growth performance and crop yields. Furthermore, their findings indicated that microbial inoculation could lead to an increase in potassium content in pea seeds over time, further emphasizing the long-term benefits of microbial applications in sustainable agriculture.

Chlorophyll content of peas (*Figure 16/E*), a key indicator of photosynthetic efficiency and overall plant health, was found to be higher in the A- (loosening with no microbes) (maximum average 20.33 mg/100 g) and B- (no-tillage no microbes) treatments. These elevated values indicate that conservation tillage practices, characterized by minimal soil disturbance, may positively influence chlorophyll synthesis and thus enhance the photosynthetic capacity of pea plants. This effect is likely due to improved soil moisture retention, better root development, and reduced physiological stress under reduced tillage conditions. These findings are consistent with the study by Rangappa et al. (2024), which reported that pea plants grown under reduced tillage and residue retention exhibited significantly higher photosynthetic rates, leaf relative water content, and stomatal conductance compared to those under conventional tillage and treatments without residue retention. The evidence supports the conclusion that conservation-oriented soil management practices can contribute to enhanced physiological performance and productivity in legumes such as peas.

Starch content of peas (*Figure 16/F*) exhibited variability across the different treatments, with the highest concentrations observed in the A (loosening) treatments (maximum average 7.47%). In contrast, the B (no-tillage) treatments revealed slightly lower starch levels. This result suggests that the relationship between microbial activity and starch accumulation is complex, potentially influenced by the interplay between soil disturbance and nutrient availability. While microbial inoculation may enhance certain physiological processes, its impact on starch synthesis appears less direct and possibly mediated by broader environmental and soil conditions. Interestingly, these findings are somewhat contrasted by the work of Nikolopoulou et al. (2007), who reported that starch content in peas remained largely unaffected by different tillage systems or microbial treatments. Their study emphasized that, unlike other compositional traits, starch levels were relatively stable across varying management practices. However, they did identify environmental factors, such as drought stress and temperature fluctuations, as significant influences on starch accumulation. Notably, severe drought conditions were associated with increased protein concentrations in pea seeds, which may indirectly impact starch deposition. Additionally, variations in temperature and rainfall patterns during critical growth stages were found to affect overall starch composition and accumulation in pea crops, underscoring the role of climatic conditions in shaping seed quality attributes.

Total sugar content of peas (*Figure 16/G*) remained relatively low across all treatments, with values ranging from 1.1% in the A+ (loosening with microbes) treatment to a maximum average of 1.53% in the B- (no-tillage no microbes) treatment. Interestingly, the no-tillage treatments, particularly B-, exhibited slightly higher sugar concentrations, which may be attributed to improved carbon availability and enhanced root development in minimally disturbed soils. Notably, total sugar content was the only parameter to show a statistically significant difference

($p < 0.05$) among the treatments, underscoring the relevance of this trait in differentiating soil management strategies. To further investigate this variation, a one-way ANOVA was conducted, followed by a post hoc Tukey HSD test. The results confirmed a statistically significant difference between treatments A+ and B- (corresponding to treatment A and treatment D, respectively, explained below). This treatment is loosening with microbes compared to the no-tillage no microbes. This highlights a noteworthy divergence in sugar accumulation linked to tillage and microbial application. The highest sugar concentration was observed in the B-1 treatment. These findings are supported by the study of Nikolopoulou et al. (2007), who reported that environmental variables such as, cultivation year, geographic location, and their interactions significantly influenced soluble sugar levels, particularly sucrose, in pea seeds. Their research emphasizes the sensitivity of sugar metabolism to external factors, which may interact with soil management practices. Interestingly, the lower sugar content observed in the A+ treatment (involving microbial inoculation and soil loosening) may suggest that microbial activity influences carbohydrate allocation. One possible explanation is that microbial inoculants enhance nitrogen uptake or promote protein synthesis, thereby redirecting carbon from soluble sugar accumulation toward amino acid and protein pathways. Alternatively, microbial interactions may alter hormonal balances or enzymatic activities that regulate sugar metabolism in developing seeds.

As the significant difference was found in the case of **total sugar content of peas**, consequently, a post-hoc analysis was conducted using the Tukey HSD Test via the ASTATSA online calculator to identify which specific treatment pairs differed significantly.

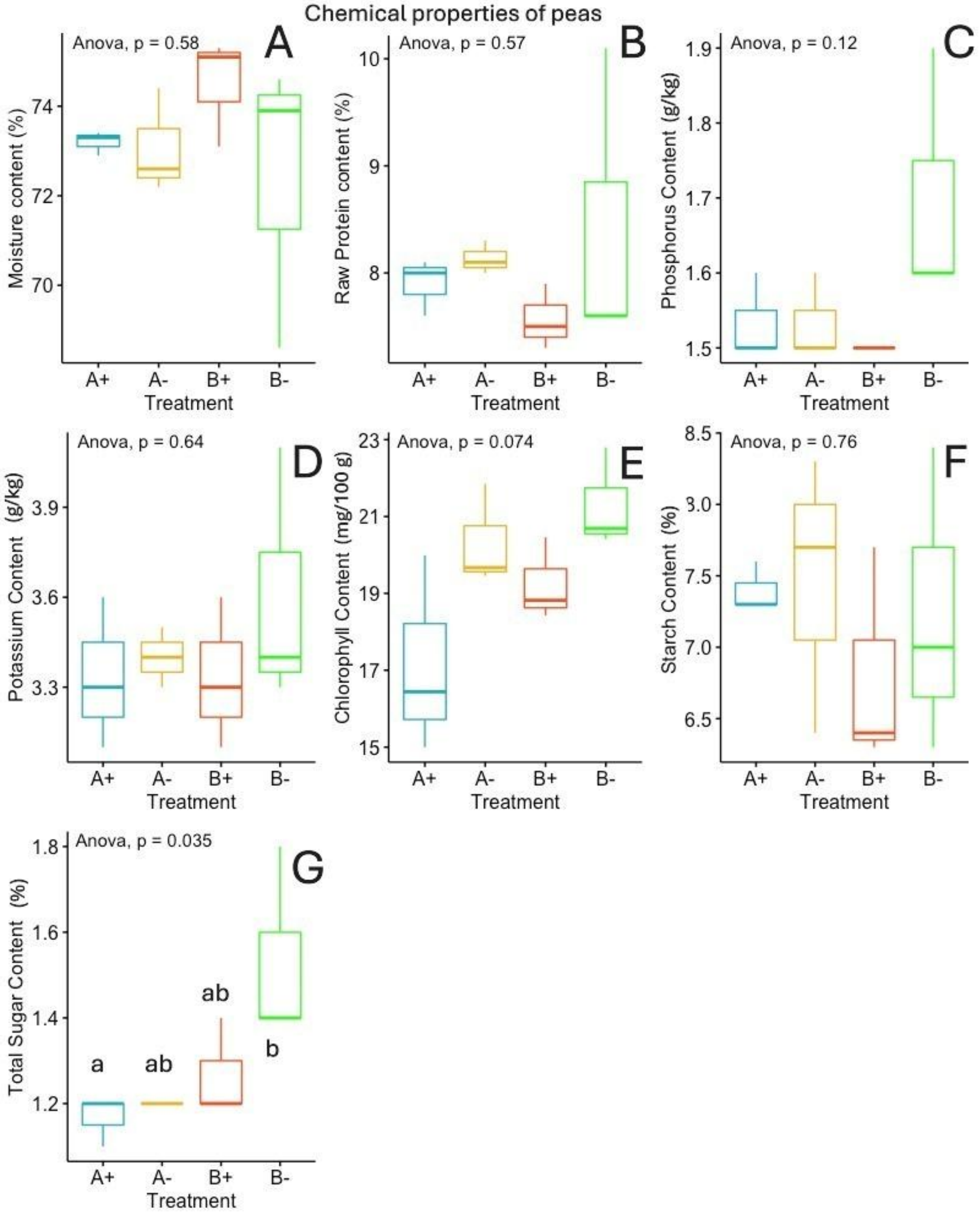
For clarity, the treatments are denoted as follows:

- A+ = A
- A- = B
- B+ = C
- B- = D

The Tukey HSD results revealed a significant difference between treatments A+ and B- (i.e., treatment A and treatment D), treatments with loosening with microbes and treatments with no-tillage no microbes, indicating that these two groups differed notably in terms of total sugar content. The complete results of the Tukey HSD test are presented in *Figure 32.* (presented in Appendices).

Figure 16 presents box plot visualizations summarizing the chemical properties of peas. The figure is based on statistical analyses conducted using one-way ANOVA implemented in the R statistical environment.

Figure 16: Summary of the chemical properties of peas with p-values (II year-2024)



** p-value > 0.05-no significant differences between treatments; p-value < 0.05-significant differences between treatments*
***The letters a, b, and ab show statistical groupings: treatments sharing the same letter (e.g., a or b or ab) are not significantly different from each other, while treatments with different letters represent significantly different groups*
A-: Loosening (No microbes); B-: No-tillage (No microbes); A+: Loosening with Microbes; B+: No-tillage with Microbes

Source: Own work

Tables 11 and *Table 12* summarize the chemical composition of lettuce under similar treatment conditions (A-, B-, A+, and B+). The analyses were conducted for both air-dry and wet (original) samples. The parameters measured include moisture content, phosphorus content, calcium content, potassium content, magnesium content, and iron content. Each table reports the average of three replicates for each of the four treatments.

Table 11: Laboratory results for chemical properties of lettuce (Air-dry sample) (III year-2025)

Treatment	Moisture Content %	Phosphorus Content g/kg	Calcium Content (g/kg)	Magnesium Content (g/kg)	Potassium Content (g/kg)	Iron Content(mg/kg)
A-	9.1	3.25	23.7	5.70	12.6	1243
B-	8.5	2.40	19.7	4.46	12.4	1044
A+	8.8	3.00	19.8	4.50	11.8	1922
B+	7.8	2.64	18.4	4.09	11.7	1307

A-: Loosening (No microbes); B-: No-tillage (No microbes); A+: Loosening with Microbes; B+: No-tillage with Microbes

Source: Own work

Table 12: Laboratory results for chemical properties of lettuce (Original (wet) sample) (III year-2025)

Treatment	Moisture Content %	Phosphorus Content g/kg	Calcium Content (g/kg)	Magnesium Content (g/kg)	Potassium Content (g/kg)	Iron Content(mg/kg)
A-	79.8	0.722	5.26	1.27	2.80	276
B-	80	0.523	4.29	0.972	2.70	228
A+	79.1	0.690	4.55	1.04	2.71	442
B+	74.5	0.734	5.10	1.13	3.24	362

A-: Loosening (No microbes); B-: No-tillage (No microbes); A+: Loosening with Microbes; B+: No-tillage with Microbes

Source: Own work

No statistically significant differences were observed in the chemical properties of lettuce (both for dry and wet samples). The following discussion focuses on numerical differences observed in the data and the patterns suggested, highlighting the potential influence of microbial inoculation and different soil management practices on the chemical properties of lettuce, for both air-dried and wet samples.

As for **moisture content of lettuce**, the highest average measured moisture content in laboratory was 9.1% *(Figure 17/A)* in the A- treatment (loosening without microbes) for air-dry samples, and 80% *(Figure 18/A)* in the B- treatment (no-tillage without microbes) for wet (original) samples. Literature values for air-dried lettuce samples generally range from 8-10% moisture content (literature). The highest value observed in this study (9.1%) for the A- treatment aligns well within this commonly reported range. In the literature, fresh samples typically show moisture contents in the range of 93-96% (Olatayo Jekayinfa et al., 2023). In this study, however, the highest moisture value obtained for wet samples (80% in the B- treatment) was lower than what has been reported previously. This discrepancy may be due to partial sample dehydration prior to analysis, differences in handling or storage conditions, or specific environmental or varietal characteristics unique to the study. Although the values do not fully match the literature, they remain within a reasonable range and do not compromise the validity of the findings, as the pattern and comparative differences among treatments are still demonstrated.

The highest **phosphorus content of lettuce** measured in this study was 3.25 g/kg in the air-dried samples (*Figure 17/B*) for the A- treatment (loosening without microbes), and 0.734 g/kg in the wet samples (*Figure 18/B)* for the B+ treatment (no-tillage with microbial inoculation). Literature values for air-dried lettuce typically range from 0.98 to 2.27 g/kg, depending on the level of fertilization and environmental factors (Abdul et al., 2012). In this study, it has been measured maximum of 3.25 g/kg is notably higher than most published values, even exceeding the levels observed with high phosphorus fertilization. This may indicate either particularly high nutrient uptake in this treatment, differences in soil or fertilizer application, or the impact of experimental conditions on phosphorus accumulation. For wet (fresh) lettuce samples, phosphorus content in literature is generally reported in the range of 0.25 to 0.28 g/kg (Broadley et al., 2003; Petek et al., 2020). The highest value in this study (0.73 g/kg) is substantially above the range most commonly noted in published research. This suggests that this experiment promoted enhanced phosphorus uptake. The phosphorus contents in both air-dried and wet samples from this study exceed values commonly found in the literature. Possible reasons for this observation may include the experimental design and treatment effects, variations in fertilization strategies, or specific characteristics of lettuce varieties used. These higher concentrations warrant further exploration, indicating a significant response of lettuce to the conditions outlined in this research protocol.

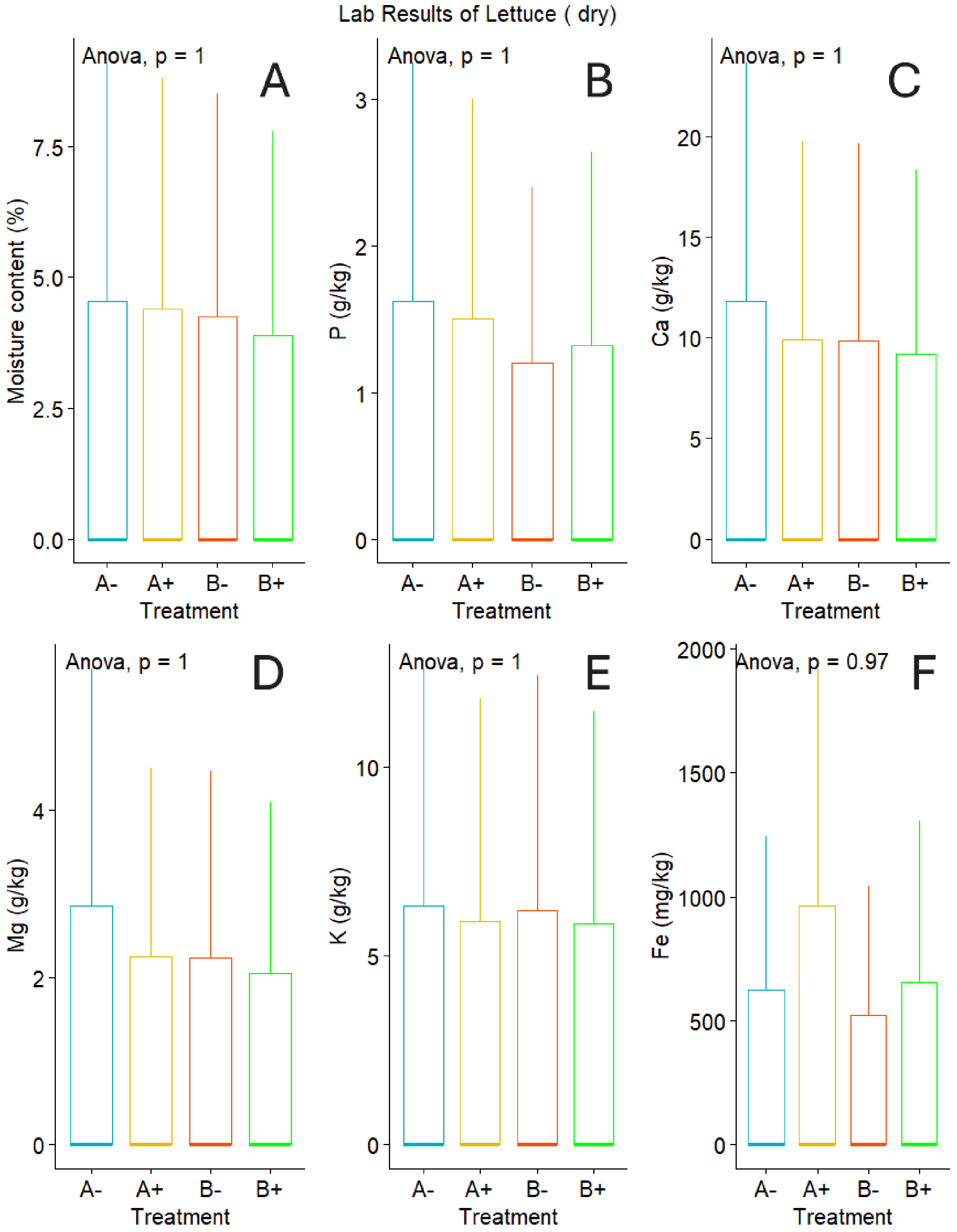
The research findings regarding **calcium content of lettuce** are well aligned with existing literature, validating the results within the wider scientific context. Literature reports that calcium concentrations in lettuce dry matter typically range from approximately 8 to 24 g/kg, with variation depending on cultivar and environmental conditions (Barta and Tibbitts, 2000; Koudela, 2008; Osman et al., 2024). The study's highest recorded calcium value in the A- treatment (loosening without microbes) of 23.7 g/kg dry matter (*Figure 17/C*) thus falls at the upper spectrum of reported values, indicating significant calcium accumulation under the applied field conditions and treatments. Furthermore, calcium content expressed on a wet basis tends to be substantially lower due to dilution by leaf moisture, which for lettuce often exceeds 85%. The wet sample (*Figure 18/C*) calcium concentration measured at A- treatment again, at 5.26 g/kg is consistent with this concept, reflecting the impact of lower moisture content on concentration levels. On the fresh weight basis, calcium contents in lettuce typically range from approximately 0.2 to 0.7 g/kg in many studies, reflecting the dilution effect due to high leaf moisture content. Wet sample from this research value of 5.26 g/kg is relatively higher, which likely correlates with the lower moisture levels observed in these samples (explained above), an aspect that has also been highlighted in literature emphasizing the inverse relationship between moisture content and nutrient concentration on a fresh weight basis. The study supports its methods by placing its findings within the context of known ranges and physiological principles found in existing literature. It emphasizes how soil and microbial amendments impact calcium uptake in lettuce. This connection strengthens the understanding of calcium nutrition in leafy vegetables and offers a useful framework for ongoing research in plant mineral nutrition.

The highest **magnesium concentrations of lettuce** observed in treatment A- (loosening without microbes), 5.7 g/kg dry matter (*Figure 17/D*) and 1.27 g/kg wet basis (*Figure 18/D*), align well with literature values for lettuce. Published research reports magnesium concentrations in dry lettuce ranging from 3.88 to 6.67 g/kg dry matter, with the present study's dry sample value of 5.7 g/kg falling within this established range (Koudela, 2008). On a fresh weight basis, the wet sample magnesium content of 1.27 g/kg exceeds typical reported values of 0.11 to 0.41 g/kg, suggesting either lower moisture content in the sampled lettuce or enhanced magnesium accumulation under the loosening treatment. The elevated fresh weight magnesium concentration indicates adequate magnesium uptake and accumulation in plant tissues, which is necessary for normal physiological processes in lettuce (Koudela, 2008). The relatively higher magnesium concentration in the A- treatment despite the absence of microbial inoculants suggests that mechanical soil loosening alone effectively improves magnesium availability. Loosening enhances soil aeration, root penetration, and water drainage, facilitating magnesium mobilization from soil reserves and uptake by plant roots. This differs from potassium dynamics, where microbial inoculants proved more effective, as magnesium availability may be less dependent on biological solubilization processes. In summary, the magnesium concentrations in treatment A- demonstrate adequate mineral nutrition status for lettuce, with values consistent with or exceeding typical literature ranges, indicating that mechanical soil loosening successfully promotes magnesium accumulation in field-grown lettuce.

The **potassium concentrations of lettuce** observed in the present study: 12.6 g/kg dry matter (*Figure 17/E*) in the A- treatment (loosening without microbes) and 3.24 g/kg wet basis (*Figure 18/E*) in the B+ treatment (no-tillage with microbes), are lower than the typical literature range of 21 to 65 g/kg dry matter reported for lettuce. This disparity reflects the differential effectiveness of soil management practices in mobilizing potassium availability (Gao et al., 2022; Koudela, 2008). Mechanical soil loosening without microbial amendment, as in treatment A-, appears insufficient for substantial potassium mobilization. Approximately 90 to 98% of soil potassium exists in unavailable forms bound within mineral structures, requiring biological or chemical processes for release. Microbial inoculants, particularly potassium-solubilizing bacteria, facilitate potassium release through organic acid production and chelation, increasing plant uptake efficiency (http 7, 2026). The B+ treatment (no-tillage with microbes) yielded 3.24 g/kg wet basis potassium, which aligns with expected fresh weight concentrations and reflects the synergistic benefit of microbial inoculation. No-tillage systems promote microbial activity and nutrient cycling, enhancing potassium solubilization compared to loosening practices. Research confirms that microbial inoculants increase potassium content in lettuce leaves by 10 to 12% through improved solubilization and root activity (Górski et al., 2017; Pagliarini et al., 2023). Although no statistically significant differences were observed among treatments, numerical differences and difference in means in the results suggested a pattern in which the use of microbial inoculants in combination with conservation tillage was associated with higher potassium uptake compared with mechanical loosening alone.

The **iron concentrations of lettuce** observed in treatment A+ (loosening with microbes), 1922 mg/kg dry matter (*Figure 17/F*) and 442 mg/kg wet basis (*Figure 18/F*), substantially exceed typical literature values for lettuce. Standard literature reports iron concentrations in dry lettuce ranging from 50 to 177 mg/kg dry matter, with optimal values at approximately 96.6 to 117.1 mg/kg (Da Silva et al., 2024). The observed dry matter iron content of 1922 mg/kg is approximately 10 to 20 times higher than these typical ranges, indicating exceptional iron accumulation. The wet sample iron content of 442 mg/kg similarly exceeds reported fresh weight values of approximately 8.6 to 262 mg/kg (Nicolle et al., 2004). This elevated concentration suggests either substantial iron availability in the soil or enhanced iron uptake mechanisms promoted by the loosening and microbial inoculation treatment. The combination of mechanical soil loosening with microbial inoculants appears highly effective in promoting iron mobilization and plant uptake. Beneficial microorganisms, particularly iron-solubilizing bacteria, produce organic acids and siderophores that solubilize bound iron and enhance bioavailability. Simultaneously, soil loosening improves aeration and root penetration, facilitating iron uptake from the rhizosphere. The notably high levels of iron in treatment A+ may reflect either the availability of iron in the soil at the site or effective biofortification achieved through the combination of soil loosening and microbial treatment. These results indicate that the use of both mechanical and biological soil amendments can significantly boost iron content in lettuce, possibly surpassing the biofortified lettuce values of 137 to 177 mg/kg dry matter reported in existing studies (Koudela, 2008).

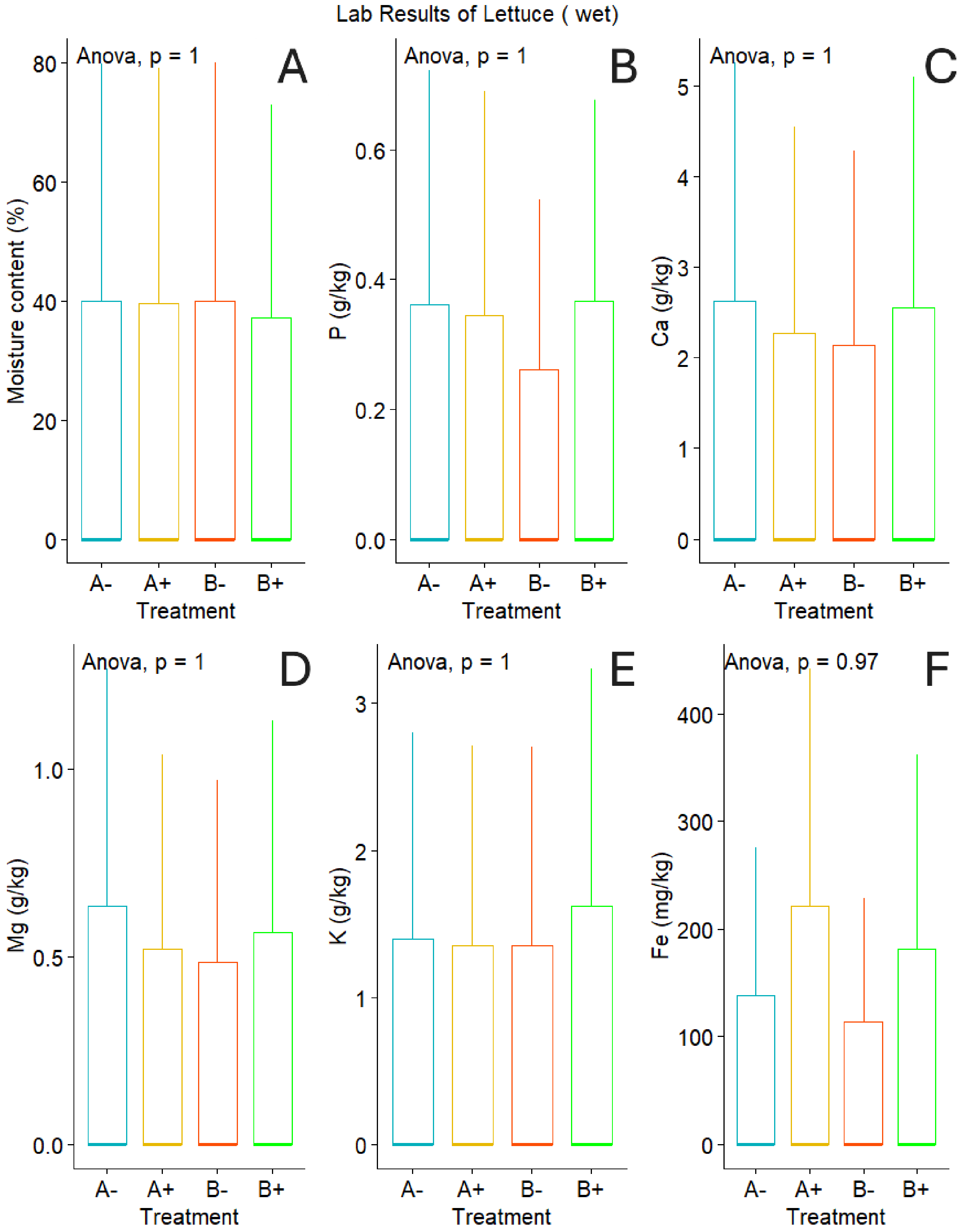
Figure 17: Summary of the chemical properties of lettuce (Dry) with p-values (III year-2025)



** p-value > 0.05-no significant differences between treatments; p-value < 0.05-significant differences between treatments*
A-: Loosening (No microbes); B-: No-tillage (No microbes); A+: Loosening with Microbes; B+: No-tillage with Microbes

Source: Own work

Figure 18: Summary of the chemical properties of lettuce (Wet) with p-values (III year-2025)



** p-value > 0.05-no significant differences between treatments; p-value < 0.05-significant differences between treatments*
A-: Loosening (No microbes); B-: No-tillage (No microbes); A+: Loosening with Microbes; B+: No-tillage with Microbes
Source: Own work

3.3. Soil Analysis After Each Planting Year: Potato, Peas, and Lettuce

The soil samples collected after potato cultivation have been organized into three distinct tables, each presenting insights into the soil's chemical properties. These measurements are categorized across four distinct cultivation conditions: A- (loosening and no microbes), B- (no-tillage and no microbes), A+ (loosening with microbes), and B+ (no-tillage with microbes). This comparative structure enables an analysis of how different farming practices and microbial activity influence soil chemistry.

Table 13 offers an in-depth examination of the soil's chemical properties, including soil plasticity (according to Arany), organic matter content, concentrations of P_2O_5, K_2O, and $CaCO_3$, as well as pH (measured in KCl solution).

Table 14 focuses on a broader range of elements, detailing the concentrations of magnesium (Mg), zinc (Zn), copper (Cu), manganese (Mn), combined nitrite and nitrate nitrogen ($(NO_2^- + NO_3^-)$-N), and sodium (Na).

Table 15 explores additional parameters, including sulfate (SO_4^{2-}), water-soluble salts, and pH (measured in H_2O solution).

Table 13: Laboratory results for chemical properties of soil, 1/3. (Potato I year-2023)

Treatment	Soil Plasticity According to Arany	Organic Matter (%)	P_2O_5 (mg/kg)	K_2O (mg/kg)	$CaCO_3$ (m/m%)	pH(KCl)
A-	37.00	4.46	1583.33	229.00	2.89	7.06
B-	38.00	4.93	2043.33	320.33	0.83	7.21
A+	33.00	4.31	1624.00	240.67	1.98	6.67
B+	35.67	4.29	1881.67	174.67	1.41	7.16

A-: Loosening (No microbes); B-: No-tillage (No microbes); A+: Loosening with Microbes; B+: No-tillage with Microbes

Source: Own work

Table 14: Laboratory results for chemical properties of soil, 2/3. (Potato I year-2023)

Treatment	Mg (mg/kg)	Zn (mg/kg)	Cu (mg/kg)	Mn (mg/kg)	$(NO_2^- + NO_3^-)$-N (mg/kg)	Na (mg/kg)
A-	165.67	29.20	8.24	71.83	19.53	48.10
B-	181.67	29.70	7.72	76.60	19.27	57.73
A+	152.67	25.47	6.97	84.67	14.03	54.30
B+	164.00	29.53	9.47	82.77	17.13	53.80

A-: Loosening (No microbes); B-: No-tillage (No microbes); A+: Loosening with Microbes; B+: No-tillage with Microbes

Source: Own work

Table 15: Laboratory results for chemical properties of soil, 3/3. (Potato I year-2023)

Treatment	SO_4^{2-} (mg/kg)	All Water-Soluble Salts (m/m%)	pH(H_2O)
A-	49.13	<0.02	7.24
B-	60.83	<0.02	7.46
A+	45.90	<0.02	7.02
B+	54.67	<0.02	7.46

A-: Loosening (No microbes); B-: No-tillage (No microbes); A+: Loosening with Microbes; B+: No-tillage with Microbes

Source: Own work

Table 16 presents key soil parameters, including soil plasticity (according to Arany), organic matter content (%), P_2O_5 (mg/kg), K_2O (mg/kg), $CaCO_3$ (m/m%), pH (measured in KCl solution), and magnesium (Mg, mg/kg), measured after peas.

Table 17 presents supplementary parameters, including zinc, copper, manganese, nitrate and nitrite nitrogen, sodium, sulfate, total water-soluble salts, and pH (measured in H_2O solution), measured after peas.

Table 16: Laboratory results for chemical properties of soil, 1/2. (Peas II year-2024)

Parameter	A-	B-	A+	B+
Soil plasticity according to Arany	34.33	35.00	31.33	32.67
Organic matter (%)	3.92	4.19	3.54	3.79
P_2O_5 (mg/kg)	1412.33	1249.67	1329.67	1465.33
K_2O (mg/kg)	155.33	109.43	151.97	120.67
pH(KCl)	7.00	6.98	6.84	7.05
Mg (mg/kg)	189.33	200.67	174.00	194.00

A-: Loosening (No microbes); B-: No-tillage (No microbes); A+: Loosening with Microbes; B+: No-tillage with Microbes

Source: Own work

Table 17: Laboratory results for chemical properties of soil, 2/2. (Peas II year-2024)

Parameter	A-	B-	A+	B+
Zn (mg/kg)	35.37	27.93	25.87	30.23
Cu (mg/kg)	12.69	8.94	13.20	12.36
Mn (mg/kg)	97.40	84.63	90.83	96.53
($NO_2^- + NO_3^-$)-N (mg/kg)	19.73	16.07	19.60	17.30
Na (mg/kg)	31.27	27.60	26.07	30.43
SO_4^{2-} (mg/kg)	20.07	19.77	18.27	20.83
All water-soluble salts (m/m%)	<0.02	<0.02	<0.02	<0.02
pH(H_2O)	7.28	7.26	7.13	7.39

A-: Loosening (No microbes); B-: No-tillage (No microbes); A+: Loosening with Microbes; B+: No-tillage with Microbes

Source: Own work

Table 18 presents the average values of soil chemical and physical properties, including soil plasticity according to Arany, organic matter, P_2O_5, $CaCO_3$, K_2O, and pH(KCl).

Table 19 summarizes the average values of micronutrient and soluble component concentrations, including magnesium (Mg), zinc (Zn), copper (Cu), manganese (Mn), (NO_2^- + NO_3^-)-N, sodium (Na), sulfate (SO_4^{2-}), and total water-soluble salts.

Table 18: Laboratory results for chemical properties of soil, 1/2. (Lettuce III year-2025)

Parameter	A-	B-	A+	B+
Soil plasticity according to Arany	33.33	34.33	33	33.66
Organic matter (%)	2.43	3.21	2.60	2.92
P_2O_5 (mg/kg)	1296.33	1425.33	1261.67	1420.33
$CaCO_3$ (m/m%)	1.05	1.74	2.02	1.73
K_2O (mg/kg)	90.3	99.5	97.47	96.87
pH(KCl)	7.47	7.43	7.48	7.45

A-: Loosening (No microbes); B-: No-tillage (No microbes); A+: Loosening with Microbes; B+: No-tillage with Microbes

Source: Own work

Table 19: Laboratory results for chemical properties of soil, 2/2. (Lettuce III year-2025)

Parameter	A-	B-	A+	B+
Mg (mg/kg)	163.67	175	171	196.67
Zn (mg/kg)	26.83	30.2	26.43	28.43
Cu (mg/kg)	10.88	9.82	9.59	23.80
Mn (mg/kg)	82.57	81.2	94.13	97.93
(NO_2^- + NO_3^-)-N (mg/kg)	29.07	38.2	30.1	31.5
Na (mg/kg)	49.27	53	48.2	55.1
SO_4^{2-} (mg/kg)	20.83	21.6	23.83	20.33
All water-soluble salts (m/m%)	<0.02	<0.02	<0.02	<0.02

A-: Loosening (No microbes); B-: No-tillage (No microbes); A+: Loosening with Microbes; B+: No-tillage with Microbes

Source: Own work

Across most measured parameters over the three-year cropping period, no statistically significant differences were detected in soil properties, with the exception of soil plasticity (Arany) and pH(KCl). Nevertheless, the data suggested consistent patterns, as results showed non-significant, higher or lower mean values in response to the applied treatments, indicating directional responses even where statistical significance was not achieved.

For the soil properties examined, significant differences among treatments were detected for Soil Plasticity according to Arany ($p = 0.025$) and for pH(KCl) ($p = 0.023$), as determined by one-way ANOVA. To further identify which specific treatments differed, a post-hoc Tukey HSD test was performed using the ASTATSA online platform.

For reference, the treatments were coded as follows:

- A+ = A

- A- = B
- B+ = C
- B- = D

For the parameter Soil Plasticity according to Arany (*Figure 19/A*), the Tukey HSD analysis indicated a statistically significant difference between treatments A+ and B-, loosening with microbes and no-tillage no microbes, suggesting that these two groups showed notable variation in both parameters. This comparison is discussed in detail in the Discussion section.

For the pH(KCl) parameter in the soil (*Figure 19/F)*, the Tukey HSD analysis showed the statistically significant difference between the A+ and B+ (loosening with microbes, no-tillage with microbes), and also between A+ and B- (loosening with microbes and no-tillage no microbes). This comparison is discussed in detail in the Discussion section.

The complete set of Tukey HSD results is presented in *Figure 33* and *Figure 34* available in Appendices.

The use of double letters (AA, BB, CC, etc.) in the figures indicates that the figure is presented in more parts. Single letters (A, B, C, etc.) refer to soil parameters shown in the first part of the figure *(*for example *Figure 19)*, while double letters (AA, BB, CC, etc.) correspond to different soil parameters displayed in the second part *(Figure 20).* This labeling scheme was adopted because a large number of soil parameters were analyzed, requiring the figure to be divided into multiple panels for clarity.

Following potato planting in 2023 (*Figure 19/A*), the chemical properties of soil showed notable variation across management practices. **Soil plasticity, expressed through Arany values**, averages ranged between 33 and 38. According to the study by Dobos et al. (2023), soil's plasticity, based on Arany's classification, typically corresponds to sandy loam textures with values ranging from 30 to 37. In this study, the A- and B- soils (no microbial application) displayed higher plasticity values compared to A+ and B+ (with microbial application) soils, which underwent loosening and no-tillage with the application of microbes. This pattern suggests that tillage and microbial activity may contribute to reducing soil plasticity, potentially improving soil structure and aeration. This parameter showed a statistically significant difference according to the Tukey HSD test. Based on the Tukey HSD analysis for the Soil Plasticity parameter according to Arany, there's a clear and significant difference between treatments A+ (loosening with microbes) and B- (no-tillage no microbes). It shows that the groups involving microbes and the no-tillage no microbes treatment displayed notable variations in both aspects. After peas in 2024 (*Figure 22/A*), soil plasticity values remained relatively stable across treatments, averages ranging from 31 to 35, again aligning with the sandy loam category defined by Arany’s classification. Similar to the findings of Dobos et al. (2023), this consistency indicates that soil texture and structure were less affected by management practices in the pea cultivation system compared to potato. In the third year, after lettuce in 2025 (*Figure 24/A*), soil plasticity was consistent across treatments, averages ranging between 33-34, which is aligned with the sandy loam textures. After the first year, it can be emphasized that microbial application and tillage reduced soil plasticity and improved structure. This difference was statistically significant (Tukey HSD). After peas and lettuce in the third year, plasticity values remained stable and consistent, suggesting limited effects of management on soil texture over time.

Organic matter serves as a fundamental indicator of soil fertility and health, underpinning nutrient availability, microbial activity, and soil structural stability. After the first experimental year in 2023 (*Figure 19/B*), average organic matter content ranged from 4.2 to 4.9 % across the different management practices. The highest averages occurred in the B- and A- (no microbes) treatments, demonstrating that soils under no-tillage conditions retained more organic matter than those exposed to loosening or microbial inoculation during the early stage. This tendency continued into the second year in 2024 (*Figure 22/B*), with B- and A- again exhibiting higher average values, suggesting that reduced soil disturbance may help preserve organic matter by limiting oxidation and decomposition. However, when each treatment was examined separately the influence of microbial activity became evident. The inoculated variants, A+ (loosening with microbes) (3.61%) and B+ (no-tillage with microbes) (3.68%), showed higher individual organic matter contents compared to the non-inoculated A- (2.78%) and B- (3.42%) treatments. These results indicate that microbial inoculation positively contributed to organic matter accumulation, likely through enhanced root exudation, microbial biomass buildup, and more efficient nutrient cycling. Such findings are supported by previous studies emphasizing the beneficial effects of microbial inoculants. Cui et al. (2023), reported that introducing microbes alongside soil amendments improved organic matter levels, nutrient status, and enzymatic activity in contaminated soils. Similarly, Janati et al. (2023), demonstrated that inoculation with phosphate-solubilizing bacteria stimulated crop growth and increased soil organic matter through more active microbial processes. In the lettuce trial in 2025 (*Figure 24/B*), by the third year, the B- treatment (no-tillage no microbes) maintained the highest average organic matter content (3.21%), suggesting the persistence of organic matter retention under minimal soil disturbance. Overall, with minimal external inputs, the organic matter content remained relatively stable and consistent with values reported in the literature, even though no significant differences were detected between treatments. Research from Magdoff and Van Es (2021), showed that microbial activity in soil generally requires a minimum of about 2% soil organic matter (SOM). Soils with SOM below 1% typically have very limited microbial function. This research area has sufficient organic matter not only because it is based on sandy loam soils, which typically contain more than 2% SOM and naturally support strong microbial activity, but also because the garden had not undergone regular or intensive tillage practices, which can drastically reduce organic matter even in otherwise good soils.

Phosphorus pentoxide (P_2O_5) represents the oxide form of phosphorus, one of the essential macronutrients required for plant growth. In soils, phosphorus plays a critical role in energy transfer (ATP formation), root development, and overall crop productivity. Adequate phosphorus availability enhances seed formation, promotes early plant establishment, and supports photosynthetic efficiency (Dong et al., 2025). In terms of nutrient content, **phosphorus pentoxide (P_2O_5)** and **potassium oxide (K_2O),** the concentrations of essential nutrients varied across different management practices. Although fluctuations were observed, no clear pattern emerged regarding the impact of tillage or microbial presence on nutrient levels. Further analysis is necessary to fully understand the specific effects of these management practices on nutrient availability. After the first year of treatment in 2023 (*Figure 19/C*), the highest average P_2O_5 concentration was recorded in the B- (no-tillage no microbes) treatment, which also exhibited the highest average K_2O content (*Figure 19/D*). Following pea cultivation in 2024, phosphorus levels indicated variation among treatments (*Figure 22/C*). The highest average concentrations were observed with treatment B+ (no-tillage with microbes) showing the highest overall average (1465.33 mg/kg), followed by A- treatment (average content 1412.33 mg/kg). The highest average of K_2O was found in A- treatment (loosening no microbes) (155.33 mg/kg) (*Figure 22/D*). These findings align with the results of Janati et al. (2023), who showed that microbial inoculation increased both phosphorus and potassium levels in the soil, and also that these patterns are followed together-increased phosphorus and potassium. After lettuce in 2025, the highest average of phosphorus pentoxide was found in B- and B+ treatments (no-tillage without and with microbes) (1425.33 mg/kg and 1420.33 mg/kg) (*Figure 24/C*). It was followed by potassium pentoxide content where the highest average was observed in B- treatment (99.5 mg/kg) (*Figure 24/E*). While observable patterns indicate that microbial inoculation may enhance phosphorus and potassium availability, no statistically significant differences were detected across treatments. Consequently, additional long-term research is needed to verify these patterns and to better understand the mechanisms governing nutrient transformations in soil ecosystems.

Soil **pH** is a fundamental parameter influencing nutrient availability, microbial activity, and overall soil fertility (Anker et al., 2021). The ideal soil pH for optimal plant growth differs depending on the type of crop. Typically, a pH level between 6.0 and 7.5 is suitable for most plants, as this range allows for the best availability of nutrients (Food and Agriculture Organization of the United Nations, 2021). After the first year of observation in 2023 (*Figure 19/F*), pH(KCl) was another soil parameter that showed significant differences among treatments. According to the Tukey HSD test, a statistically significant difference was detected particularly between treatments A+ (loosening with microbes) and B- (no-tillage without microbes), as well as between A+ and B+. This suggests that the combination of soil loosening and microbial inoculation may have influenced soil chemical properties, potentially through enhanced organic matter decomposition and cation exchange processes. Across all management practices, pH values ranged from slightly acidic to neutral, indicating generally favorable conditions for nutrient solubility and microbial activity. Literature by Mukhametov et al. (2023), and Karlen et al. (2021), highlights that the optimal pH range for potato cultivation is between 5.2 and 5.7. When soil pH exceeds 7, essential micronutrients such as magnesium, phosphorus, boron, and zinc can form insoluble compounds, reducing their availability and hindering crop uptake. In contrast, during the second year of the experiment (pea cultivation) in 2024 (*Figure 22/E*), no significant differences in pH(KCl) were observed among treatments. Overall, soil pH remained near neutral (around pH 7), suggesting that neither soil loosening nor microbial inoculation had a major impact on soil acidity or alkalinity under the given conditions. This stable, near-neutral pH supports balanced nutrient availability and provides favorable conditions for plant growth. Soil pH measured in water [pH(H_2O)] showed no significant differences between treatments at the end of either the first or second year of observation (*Figure 21/BBB* and *Figure 22/GG*). After conducting laboratory analyses in the third year in 2025 with the lettuce samples, the highest average pH value recorded was 7.48 for the A+ treatment. Despite the lack of significant variation, a consistent numerical pattern was observed, the B+ treatment (no-tillage with microbes) tended to exhibit slightly higher pH values compared to the A- (loosening without microbes) and B- (no-tillage without microbes) treatments. This suggests that microbial inoculation may have a subtle alkalizing effect on the soil environment, which can be beneficial to the soil. According to Yang et al. (2009), microbial inoculants often include bacterial strains capable of producing alkaline metabolites, such as ammonia (NH_3), during processes like nitrogen fixation and the decomposition of organic matter. The gradual accumulation of these alkaline compounds can lead to a slight increase in soil pH over time. This observation aligns with the hypothesis that soil management practices, particularly those involving reduced tillage and microbial application, can influence soil chemical dynamics. The interaction between soil structure (plasticity), tillage intensity, and microbial activity may therefore play a role in shaping soil pH numerical patterns across seasons.

Soil chemical properties following different crops revealed distinct patterns in both macronutrients and micronutrients across the four tillage and microbial inoculation treatments. In soil after potato in 2023, peas in 2024, and lettuce in 2025, macro- and micronutrients including **magnesium (Mg), zinc (Zn), copper (Cu), manganese (Mn), nitrogen as nitrate and nitrite ((NO_2^- + NO_3^-)-N), sodium (Na), and sulfate (SO_4^{2-})** were assessed to evaluate the residual nutrient status and treatment effects.

The following section presents the status of **macronutrients** in the soil. These essential elements, including magnesium (Mg), nitrogen from nitrates (NO_2^- + NO_3^-)-N, and sulfate (SO_4^{2-}), are required by plants in significant amounts (Ippolito, 2021; Karlen et al., 2021). **Magnesium concentrations** exhibited variation across crops and treatments. After potato cultivation in 2023 (*Figure 20/AA*), average Mg levels ranged from 152.67 to 181.67 mg/kg in the upper soil profile, with the B- (no-tillage no microbes) treatment showing the highest amount of Mg. Following pea harvest in 2024 (*Figure 22/F*), Mg concentrations in soil increased substantially, with average values ranging from 174 mg/kg to 200.67 mg/kg in B- (no-tillage no microbes) treatment. This pattern aligns with the well-documented nitrogen-fixing capacity of legumes, which can improve overall soil nutrient cycling and create favorable conditions for nutrient retention. After lettuce in 2025 (*Figure 25/AA*), Mg levels stayed elevated, averages ranging from 163.67 to 196.67 mg/kg (in B+ (no-tillage with microbes) treatment), maintaining relatively high concentrations particularly in conservation tillage systems. The levels of exchangeable magnesium (Ex-Mg) in soil can vary a lot. In most farming systems, having more than 120 mg/kg of Mg is usually seen as enough for the best crop production (Ishfaq et al., 2022). Even with no significant differences observed between the treatments, exchangeable magnesium tended to increase across the crop sequence, with higher concentrations observed after pea and maintained following lettuce. This indicates that the soil Mg status improved over the course of the rotations, particularly under treatments that enhance nutrient cycling and retention.

Nitrogen availability, measured as (NO_2^- + NO_3^-)-N, showed treatment-specific responses across all three crops. After potato in 2023 (*Figure 20/EE*), average concentrations ranged from 14.03 to 19.53 mg/kg across different treatments (highest in A- (loosening with microbes) treatment). Following peas in 2024 (*Figure 23/DD*), average values ranged from 16.07 to 19.73 mg/kg, with the A- (loosening without microbes) treatment again showing elevated levels of nitrogen availability. This accumulation of available nitrogen following legume cultivation reflects the biological nitrogen fixation capacity of peas (Janati et al., 2023). The sustained nitrogen levels in conservation tillage treatments, B+ (no-tillage with microbes) and B- (no-tillage without microbes), suggest reduced nitrogen mineralization and leaching compared to loosening treatments, consistent with findings that reduced tillage decreases nitrogen losses and maintains higher soil organic matter content. After lettuce in 2025 (*Figure 25/EE*), average nitrogen availability in the soil ranged between 29.07 mg/kg and 38.2 mg/kg (in B- (no-tillage no microbes) treatment). Nitrate-nitrogen (NO_3-N) indicates the quantity of nitrogen available in the soil that plants can readily absorb. The necessary levels of this nutrient can differ among crops, but generally, it should not drop below 10 mg/kg or exceed 50 mg/kg (http 8, 2026). As there was no statistically significant difference, there is an observed numerical pattern. Available nitrogen (NO_2^- + NO_3^--N) gradually increased throughout the crop sequence. Nitrogen levels were moderate after potato, increased slightly following peas due to biological nitrogen fixation, and reached their highest values after lettuce. No-tillage treatments (B+ and B-) generally supported higher nitrogen retention compared to loosening treatments, indicating reduced nitrogen losses and improved soil nutrient conservation. Across all crops and treatments, nitrogen concentrations remained within the agronomic range considered adequate for plant growth.

Sulfate (SO_4^{2-}) concentrations remained relatively steady overall, with some variation across crops and management practices. After potato cultivation in 2023 (*Figure 21/AAA*), average sulfate levels ranged from 45.90 to 60.83 mg/kg, with the B- (no-tillage no microbes) treatment showing the highest values. Following peas in 2024 (*Figure 23/FF*), sulfate concentrations declined markedly, averaging 18.27 to 20.83 mg/kg (highest in B+ (no-tillage with microbes)). After lettuce in 2025 (*Figure 25/GG*), sulfate levels rose slightly, averages ranging from 20.33 to 23.83 mg/kg, with the highest concentration observed in the A+ (loosening with microbes) treatment. Sulfate availability decreased after pea cultivation, likely due to plant uptake and potential leaching, and then partially recovered after lettuce. Across all crops, sulfate concentrations remained within a moderate range, suggesting that soil sulfate was generally stable and responsive to crop demand and treatment effects rather than showing continuous depletion. Despite the absence of significant discrepancies from this study, Lisowska et al. (2023), reported sulfate-sulfur levels ranging from 16 to 20 mg/kg following the application of fertilizer granules. In contrast, the results obtained in the higher sulfate concentrations demonstrated a wider range, indicating that factors such as crop sequence and soil management practices had a more pronounced impact on sulfate dynamics than the treatments involving fertilizer granules alone.

The next section will outline the state of **micronutrients** in the soil. Though these nutrients are needed by plants in small quantities, they play a vital role in maintaining overall soil health (Ippolito, 2021; Karlen et al., 2021). In the next section, the focus is specifically on discussing **Zinc, Copper, Manganese, and Sodium in the soil.**

Zinc concentrations showed moderate fluctuations across crop residues and treatments. After potato in 2023 (*Figure 20/BB*), average Zn concentrations ranged from 25.47 to 29.70 mg/kg, with the B- (no-tillage no microbes) treatment exhibiting the highest values. Following peas in 2024 (*Figure 23/AA*), Zn levels increased, ranging from 25.87 to 35.37 mg/kg, with the A- (loosening without microbes) treatment showing the highest concentration. After lettuce in 2025 (*Figure 25/BB*), Zn concentrations remained relatively stable, ranging from 26.43 to 30.20 mg/kg, again highest in the B- (no-tillage no microbes) treatment. The natural concentration of zinc in soil generally ranges from 10 to 300 mg/kg, with an average of approximately 50 mg/kg (Kumar et al., 2021). Zinc levels in the soil remained within the typical natural background range and did not show large fluctuations throughout the crop sequence. A slight increase in Zn concentration occurred after pea cultivation, followed by stabilization after lettuce. This indicates that crop rotation and soil management practices influenced Zn availability, but did not lead to excessive accumulation or depletion.

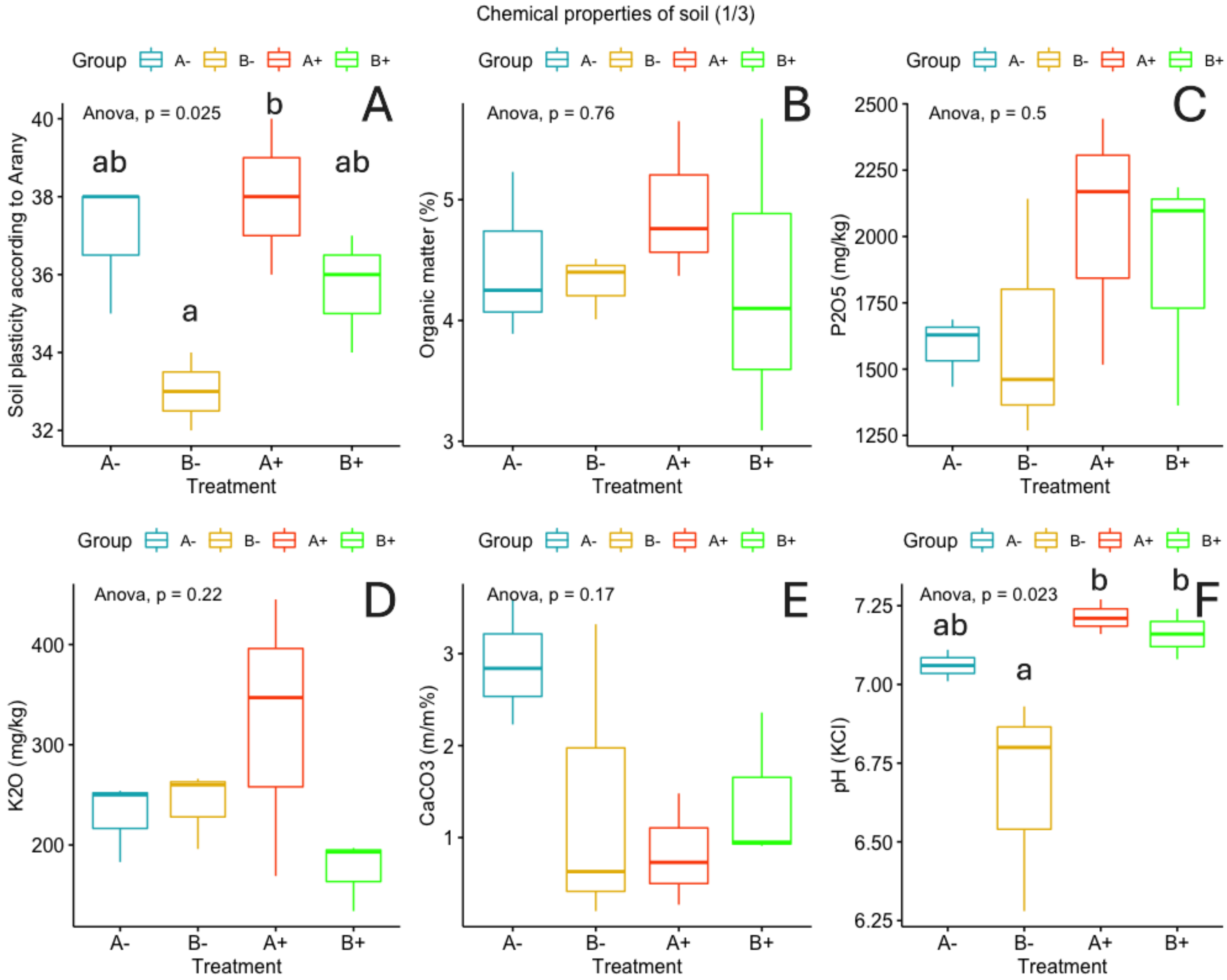
Copper concentrations showed gradual increases across the crop sequence and differed slightly among treatments. After potato in 2023 (*Figure 20/CC*), average Cu levels ranged from 6.97 to 9.47 mg/kg, with the highest concentration observed under the B+ (no-tillage with microbes) treatment. Following peas in 2024 (*Figure 23/BB*), concentrations increased to 8.94-13.20 mg/kg, with the A+ (loosening with microbes) treatment showing the highest value. After lettuce in 2025 (*Figure 25/CC*), copper levels rose further, ranging from 9.59 to 23.80 mg/kg, again highest under the B+ (no-tillage with microbes) treatment. Across seasons, treatments with microbial inoculation generally exhibited the highest Cu concentrations, although differences were not statistically significant and represent numerical differences in means rather than clear treatment effects. Globally, the typical concentration of copper in unpolluted soils ranges from 2 to 109 mg/kg, indicating that the Cu levels in this study fall well within the normal range for agricultural soils (Kumar et al., 2021). The slightly elevated copper levels in loosening or no-tillage systems receiving microbial inoculants may be related to increased organic matter accumulation at the soil surface, which enhances copper binding and retention (Kumar et al., 2021). Copper concentrations increased throughout the crop rotation, with the highest levels observed after lettuce. Treatments with microbial inoculation and reduced soil disturbance tended to retain more copper, likely due to greater organic matter inputs and improved metal complexation in the surface soil.

Manganese concentrations showed variation across crops and treatments. After potato in 2023 (*Figure 20/DD*), average Mn levels ranged from 71.83 to 84.67 mg/kg, with the highest concentration in the A+ (loosening with microbes) treatment. Following peas in 2024 (*Figure 23/CC*), Mn concentrations increased further, ranging from 84.63 to 97.40 mg/kg, with the A- (loosening without microbes) treatment exhibiting the highest value. After lettuce in 2025 (*Figure 25/DD*), Mn levels remained relatively elevated, averages ranging from 81.20 to 97.93 mg/kg, with the highest concentration observed in the B+ (no-tillage with microbes) treatment. The total manganese content in soils typically varies between 20 and 3000 mg/kg (Obeng et al., 2024). The higher Mn levels in conservation tillage treatments are consistent with research showing that reduced soil disturbance promotes organic matter accumulation at the soil surface. Increased organic matter can form complexes with manganese and influence its solubility through pH-related changes, enhancing Mn availability (Obeng et al., 2024). Manganese concentrations increased after pea cultivation and remained relatively high after lettuce. Treatments involving microbial inoculation or reduced tillage tended to maintain higher Mn levels, suggesting improved nutrient retention and greater Mn availability under conservation management.

Sodium concentrations fluctuated across the crop sequence and treatments. After potato in 2023 (*Figure 20/FF*), average Na levels ranged from 48.10 to 57.73 mg/kg, with the highest concentration observed in the B- (no-tillage no microbes) treatment. Following pea cultivation in 2024 (*Figure 23/EE*), sodium concentrations decreased substantially, averages ranging from 26.07 to 31.27 mg/kg, with the A- (loosening without microbes) treatment showing the highest value. After lettuce in 2025 (*Figure 25/FF*), sodium levels increased again, averages ranging from 48.20 to 55.10 mg/kg, with the highest concentration found in the B+ (no-tillage with microbes) treatment. According to the literature, the ideal concentration of sodium in soil is approximately 62 mg/kg, although acceptable values can vary depending on soil type and environmental conditions (Liu et al., 2021). Sodium levels decreased after peas and increased again after lettuce, returning near the initial values measured after potato. This pattern suggests that crop type and associated soil moisture dynamics influence sodium availability, while conservation tillage practices may support slightly higher sodium retention in the soil.

Figure 19, 20 and *21* presents the results from soil samples in Box Plots collected after potato cultivation.

Figure 19: Summary of the chemical properties of soil with p-values, 1/3 (Potato I year-2023)



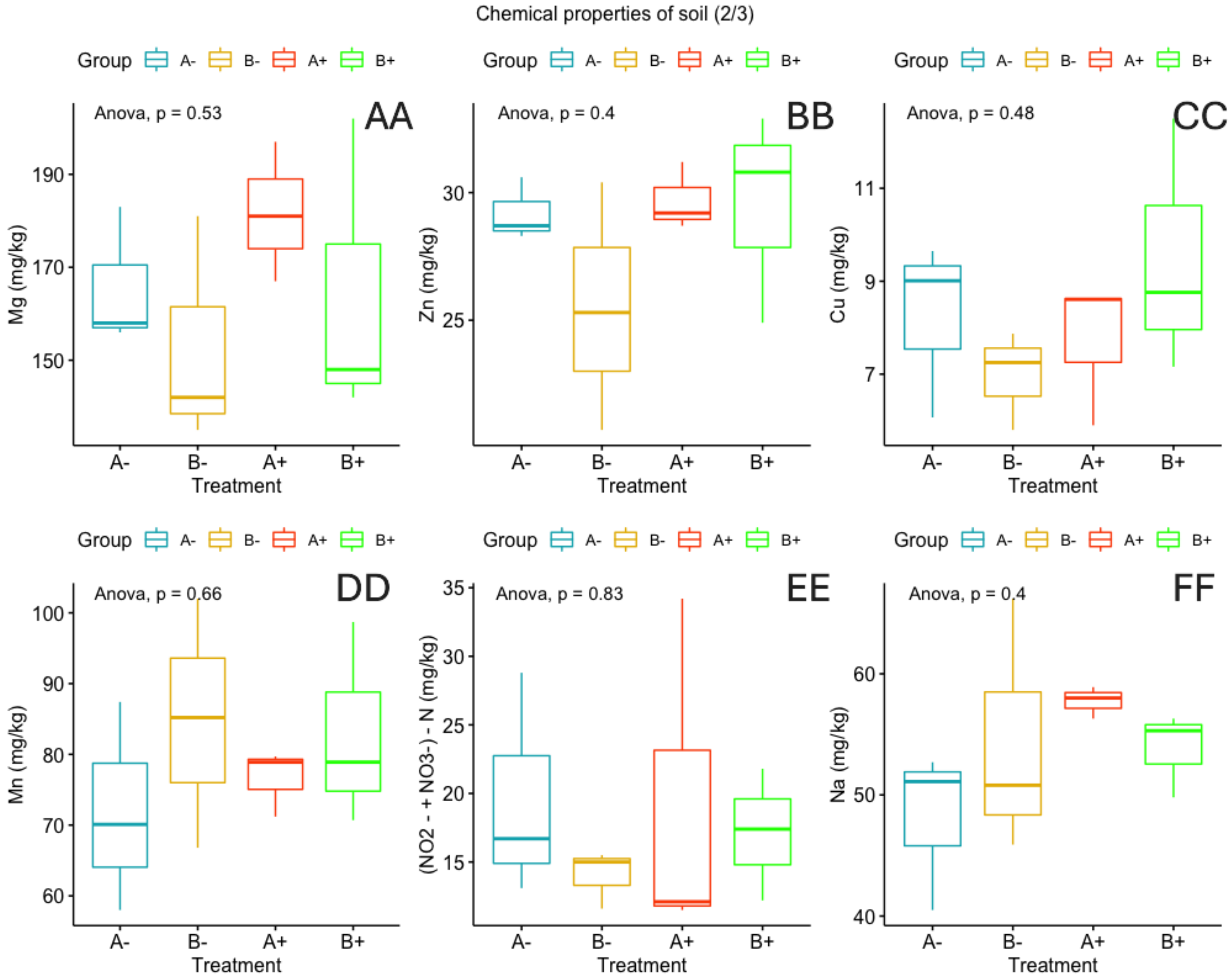
** p-value > 0.05-no significant differences between treatments; p-value < 0.05-significant differences between treatments*

***The letters a, b, and ab show statistical groupings: treatments sharing the same letter (e.g., a or b or ab) are not significantly different from each other, while treatments with different letters represent significantly different groups*

A-: Loosening (No microbes); B-: No-tillage (No microbes); A+: Loosening with Microbes; B+: No-tillage with Microbes

Source: Own work

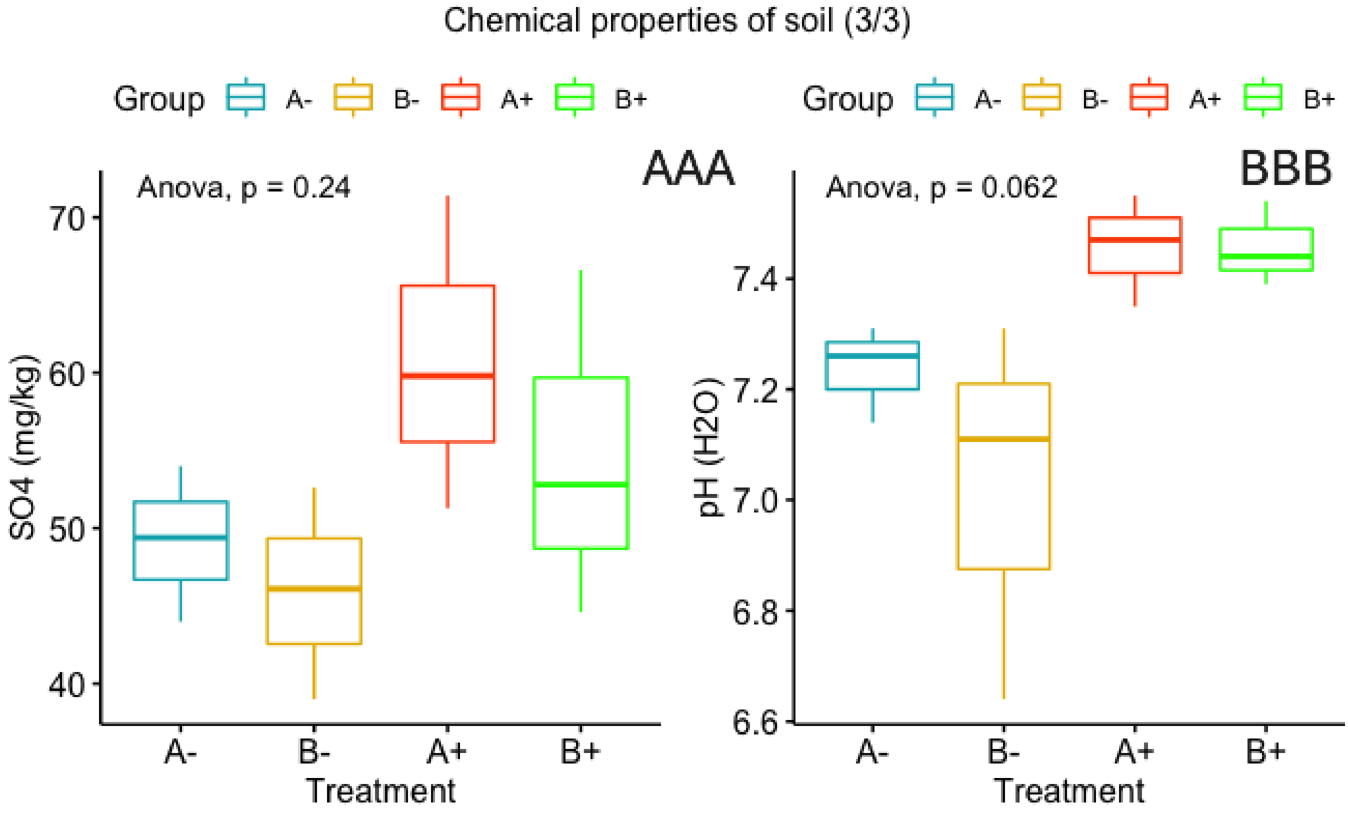
Figure 20: Summary of the chemical properties of soil with p-values, 2/3 (Potato I year-2023)



** p-value > 0.05-no significant differences between treatments; p-value < 0.05-significant differences between treatments*
A-: Loosening (No microbes); B-: No-tillage (No microbes); A+: Loosening with Microbes; B+: No-tillage with Microbes

Source: Own work

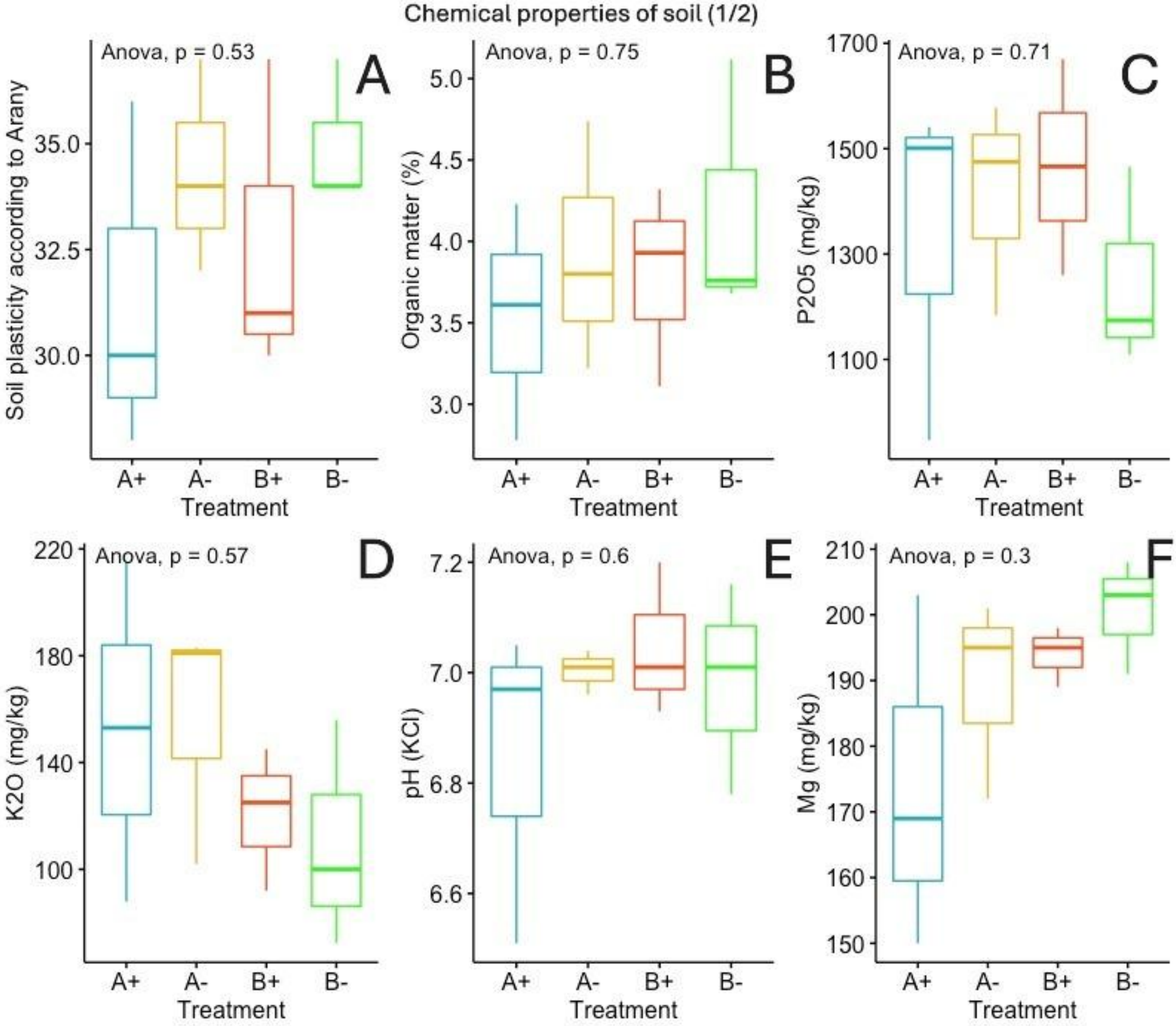
Figure 21: Summary of the chemical properties of soil with p-values, 3/3 (Potato I year-2023)



** p-value > 0.05-no significant differences between treatments; p-value < 0.05-significant differences between treatments*
A-: Loosening (No microbes); B-: No-tillage (No microbes); A+: Loosening with Microbes; B+: No-tillage with Microbes
Source: Own work

After peas, the soil sample results have been organized into two tables above, each providing an in-depth analysis of soil's chemical properties. Corresponding figures (*Figures 22* and *23*) present these parameters visually using box plots generated in R for a clear comparative analysis.

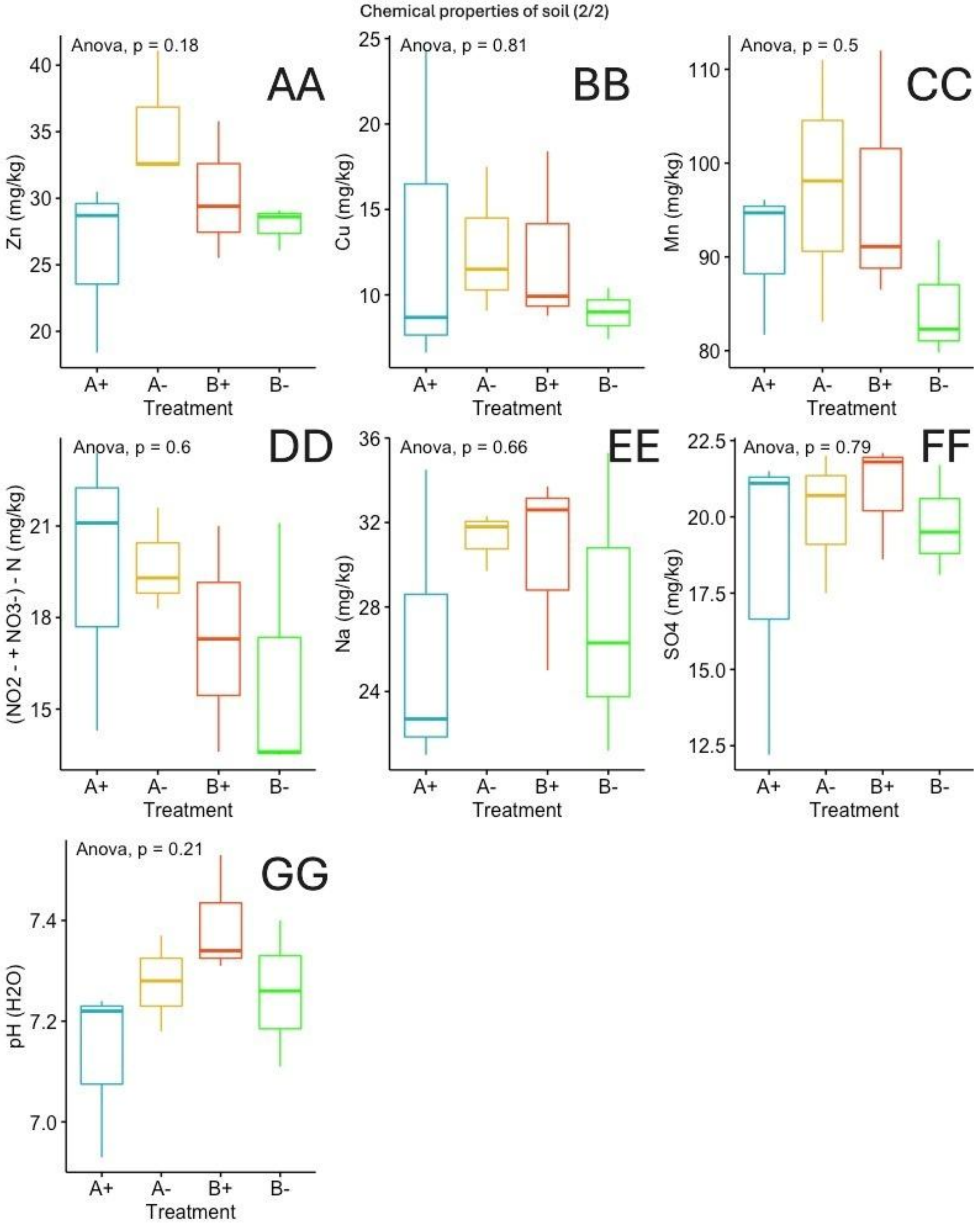
Figure 22: Summary of the chemical properties of soil with p-values, 1/2 (Peas II year-2024)



** p-value > 0.05-no significant differences between treatments; p-value < 0.05-significant differences between treatments*
A-: Loosening (No microbes); B-: No-tillage (No microbes); A+: Loosening with Microbes; B+: No-tillage with Microbes

Source: Own work

Figure 23: Summary of the chemical properties of soil with p-values, 2/2 (Peas II year-2024)

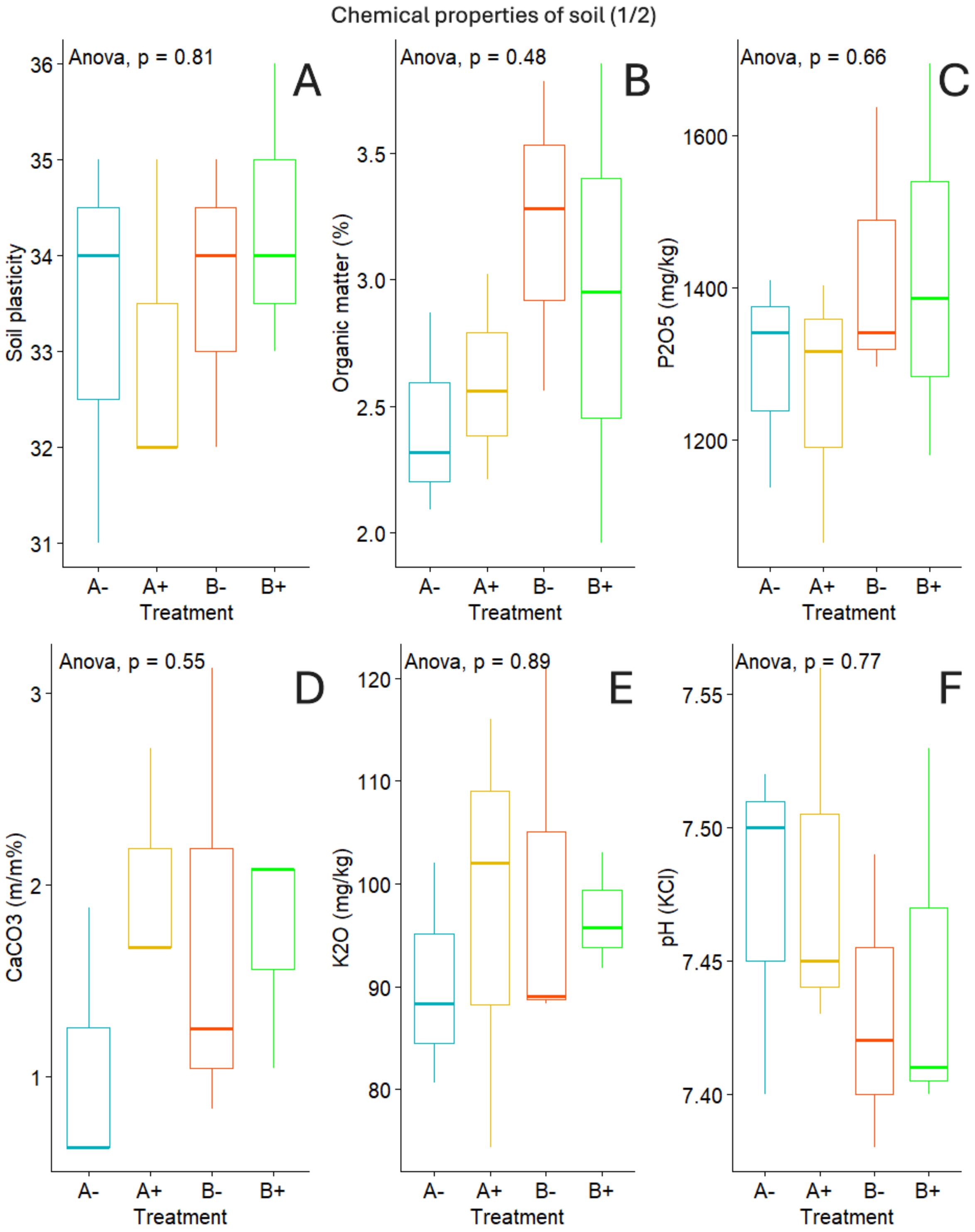


** p-value > 0.05-no significant differences between treatments; p-value < 0.05-significant differences between treatments*
A-: Loosening (No microbes); B-: No-tillage (No microbes); A+: Loosening with Microbes; B+: No-tillage with Microbes

Source: Own work

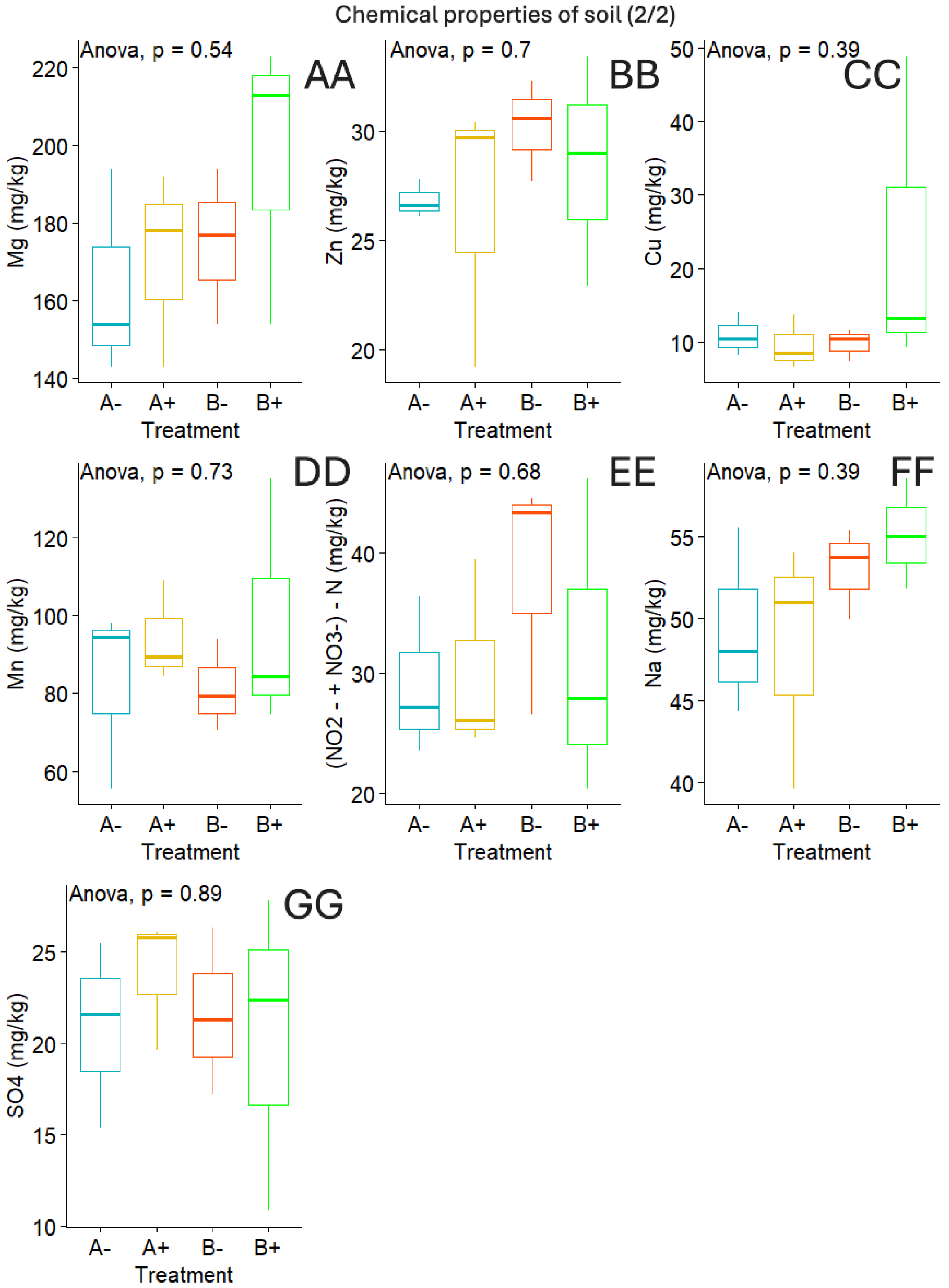
Similar to potato and peas, after lettuce cultivation, the soil sample results were organized into two tables, each providing a detailed analysis of soil's chemical properties. The corresponding figures (*Figures 24* and *25*) visually represent these parameters through box plots generated in R, offering a clear and comparative overview of the data.

Figure 24: Summary of the chemical properties of soil with p-values, 1/2 (Lettuce III year-2025)



** p-value > 0.05-no significant differences between treatments; p-value < 0.05-significant differences between treatments*
A-: Loosening (No microbes); B-: No-tillage (No microbes); A+: Loosening with Microbes; B+: No-tillage with Microbes
Source: Own work

Figure 25: Summary of the chemical properties of soil with p-values, 2/2 (Lettuce III year-2025)



** p-value > 0.05-no significant differences between treatments; p-value < 0.05-significant differences between treatments*
A-: Loosening (No microbes); B-: No-tillage (No microbes); A+: Loosening with Microbes; B+: No-tillage with Microbes
Source: Own work

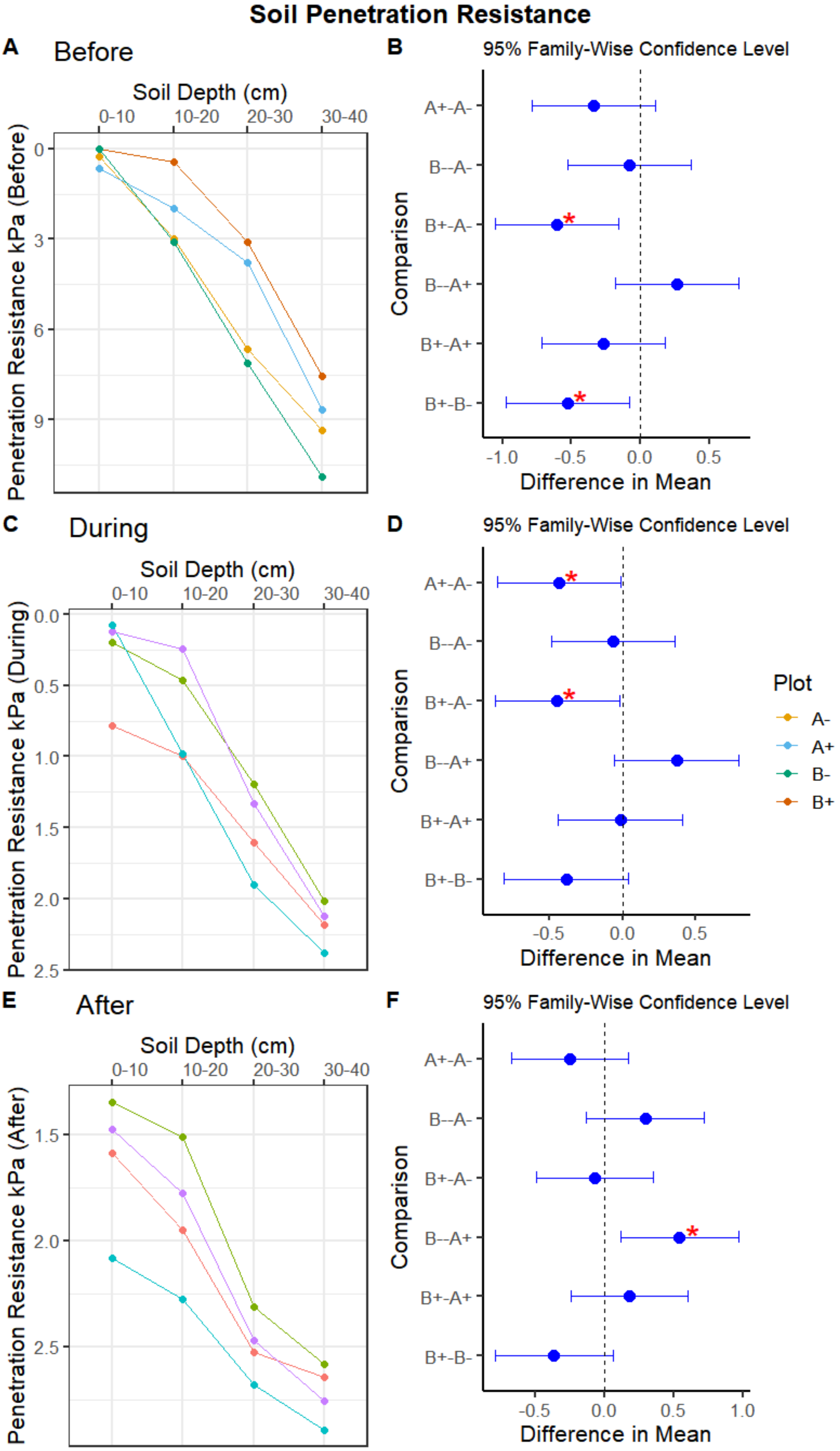
3.4. Soil Penetration, Soil Moisture, and Soil Infiltration Tests

Each figure below presents the R-generated results for soil penetration (*Figure 26*), soil moisture (*Figure 27*) and soil infiltration (*Figure 28*) tests. Bar graphs (left panels) show the mean rates for each treatment, while the accompanying Tukey pairwise comparison plots (right panels) display the differences in means with 95% confidence intervals, indicating whether any statistically significant differences exist among the treatment groups.

In March 2024, before planting of peas, penetration resistance, soil moisture and soil infiltration have been measured. In June 2024, during growth of peas, penetration resistance has been measured. In July 2024, after harvesting, again penetration resistance, soil moisture and soil infiltration have been measured.

Figure 26 presents result for soil penetration resistance measured before, during, and after planting of peas. The figure includes a comparison of mean values across these time points, highlighting changes and patterns over the course of the planting process with peas.

Figure 26: Soil Penetration Resistance Before, During, and After Planting (Peas II year-2024)



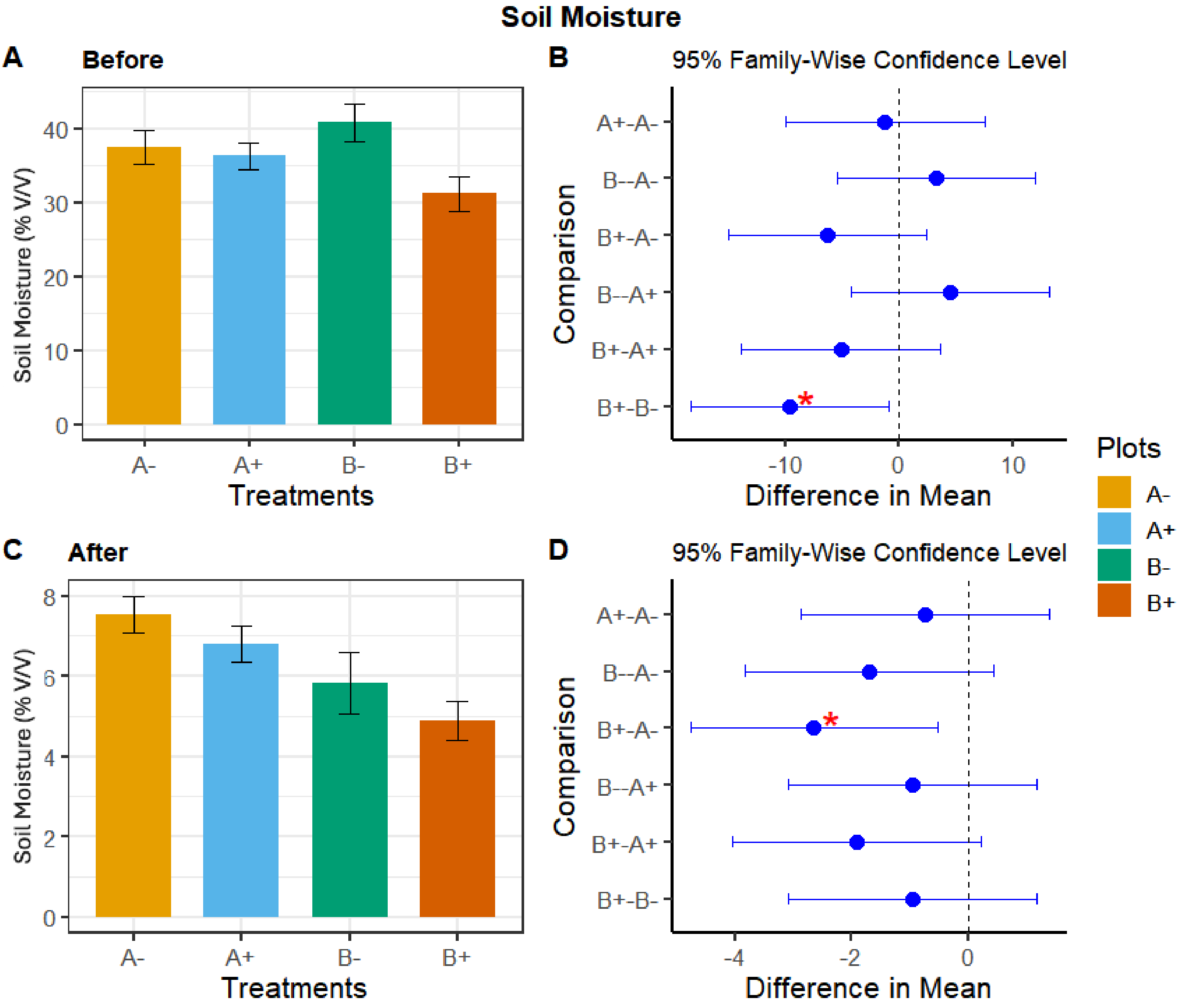
A-: Loosening (No microbes); B-: No-tillage (No microbes); A+: Loosening with Microbes; B+: No-tillage with Microbes

Source: Own work

Since significant differences were observed, as indicated by the red asterisks (*) in the comparison plots (*Figure 26/B,D,F*), a detailed statistical analysis using Tukey's Honest Significant Difference (HSD) test was conducted to further explore pairwise differences between treatments. This analysis was carried out separately for the periods before, during, and after planting of peas to capture any changes over time. The results of these comparisons, including confidence intervals and significance levels, are presented in *Tables 19, 20,* and *21,* respectively, available in Appendices. These tables provide a clearer understanding of where significant differences occurred among the treatments at each stage.

Soil moisture was measured once during the planting of peas, using both fresh and oven-dried soil samples. The results, presented in *Figure 27,* reflect the moisture content by comparing the weight of soil before and after drying. This provides an indication of the actual soil moisture level at the time of sampling.

Figure 27: Soil Moisture Content During Pea Planting (II year-2024) Based on Fresh and Dry Soil Weights



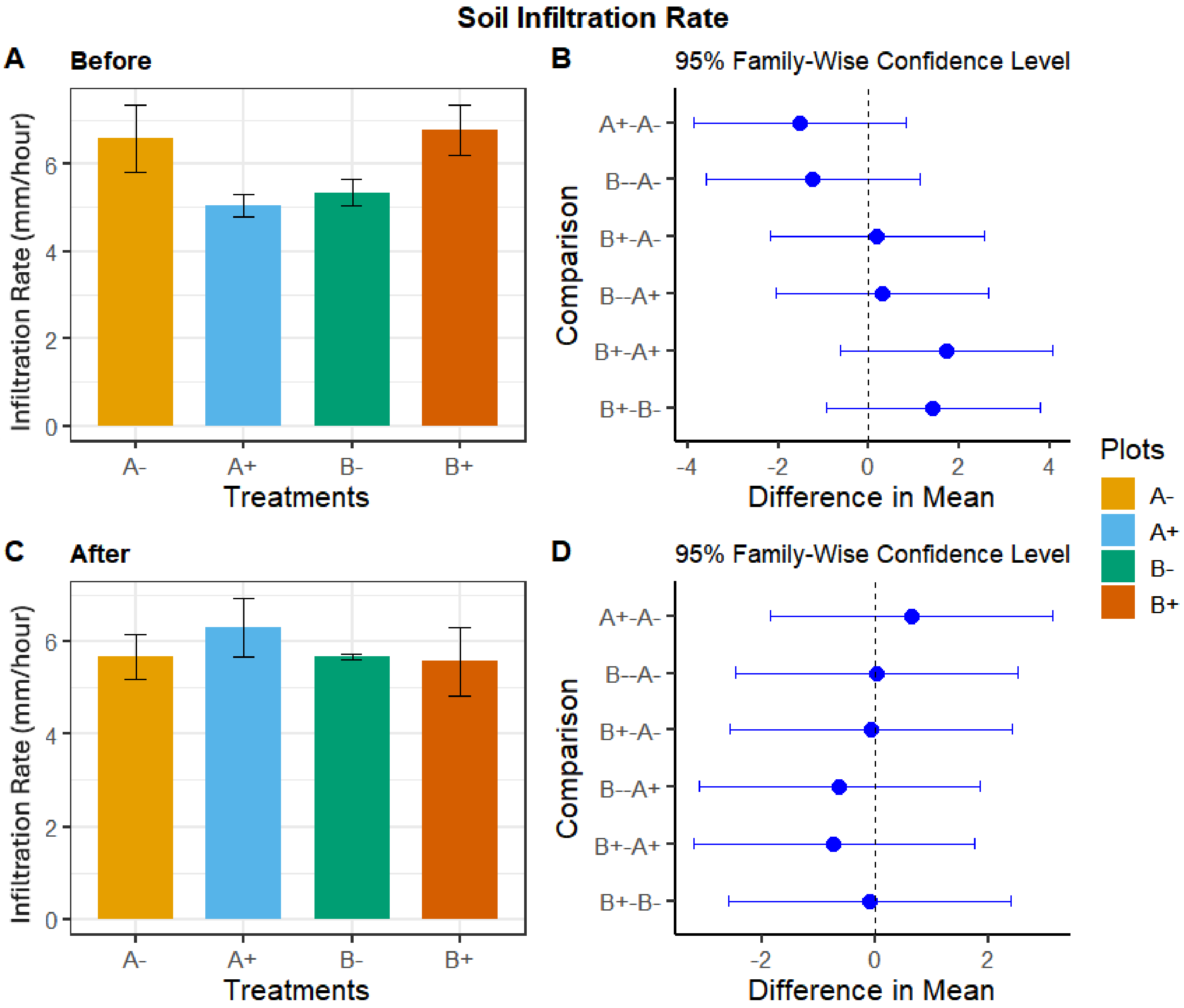
A-: Loosening (No microbes); B-: No-tillage (No microbes); A+: Loosening with Microbes; B+: No-tillage with Microbes

Source: Own work

Significant variations in soil moisture, as indicated by the red asterisks (*) in the comparison plots (*Figure 27/B,D*), prompted a detailed statistical assessment using Tukey's Honest Significant Difference (HSD) test to examine pairwise differences among treatments. This analysis was performed separately for each planting, considering both fresh and dry samples (before and after) to capture temporal changes. The outcomes of these comparisons, including confidence intervals and significance levels, are summarized in *Tables 22* and *23,* respectively, available in Appendices. These tables offer a clearer depiction of where significant differences occurred among treatments at each stage.

Figure 28 presents the soil infiltration rate measured before and after planting peas across different treatments.

Figure 28: Soil Infiltration Rate Before and After Pea Planting (II year-2024) Across Treatments



A-: Loosening (No microbes); B-: No-tillage (No microbes); A+: Loosening with Microbes; B+: No-tillage with Microbes

Source: Own work

Analysing **soil penetration (*Figure 26*)**, the application of loosening or no-tillage, paired with or without microbes, revealed considerable variation in soil penetration resistance, as indicated by Tukey HSD pairwise comparisons before, during, and after planting. Notably, the introduction of microbial inoculants, whether combined with loosening or no-tillage, frequently resulted in statistically significant differences in penetration resistance compared to their non-microbial counterparts. Loosening without microbes (A-) generally showed higher soil penetration resistance compared to loosening with microbes (A+), though this difference was not always statistically significant. The addition of microbes (A+) appeared to enhance soil structure, likely through increased biological activity that promotes aggregation and reduces compaction. No-tillage treatments (B- and B+) serve as important comparisons. B-, no-tillage without microbes often exhibited higher resistance, but integrating microbial inoculants (B+) with no-tillage significantly reduced resistance, indicating improved soil porosity and structure, perhaps due to microbe-driven processes like organic matter decomposition and stabilization. The most prominent differences, marked as significant in Tukey's tests, arose between B+ (no-tillage with microbes) and A+ (loosening with microbes), as well as between B+ and B-, highlighting the substantial effect of combining no-tillage practices with microbial amendments. These findings suggest that microbial treatments can offset negative impacts of soil compaction or lack of disturbance in no-tillage systems, leading to more favorable soil physical properties even under reduced mechanical intervention. The patterns of significance and resistance change before, during, and after planting indicate both immediate and sustained effects of management and microbial inputs. Significant improvements due to microbial inoculation persisted after planting, underlining the long-term benefits for soil health, especially in conservation tillage systems. This comparative analysis underscores the potential for integrating microbial amendments into both loosening and no-tillage management to maximize soil physical health. For practitioners, it means that microbial inoculants can be a key factor in enhancing root growth environments, reducing compaction, and supporting resilient agroecosystems, particularly where tillage reduction is part of sustainable farming goals. These results are strongly supported by findings in the soil science literature. Studies have shown that tillage interventions generally reduce soil penetration resistance, especially in the upper soil layers, enabling easier root penetration and improving soil aeration compared to no-tillage systems, which can lead to compaction if not managed properly. For example, Oduma et al. (2017), and Fasinmirin and Reichert (2011), documented that zero-tilled soils have higher penetration resistance than ploughed or harrowed soils, and that the effect of tillage on lowering resistance is often temporary, with compaction returning over time. Furthermore, recent research emphasizes the value of microbial inoculants in improving soil structure and mitigating compaction, particularly in conservation systems. Microbial practices, when combined with no-tillage or reduced tillage, have been shown to significantly lower penetration resistance and enhance soil physical properties, supporting both crop performance and long-term soil health. These alignments affirm that the observed treatment effects on soil penetration resistance in this study are consistent with established scientific patterns and describable numbers.

Concerning **soil moisture (Figure 27),** the results show statistically significant differences in soil moisture among treatments, both before and after drying, with the most notable contrasts between the B+ and B− treatments. The pairwise Tukey HSD test indicates that, before drying, the B+ treatment had significantly lower soil moisture compared to the B- treatment (difference: -9.9966, p = 0.0272), and after drying, B+ was again significantly lower than A− (difference: -2.6399, p = 0.0102) as visualized by the non-overlapping confidence intervals in the summary plots. These findings suggest that both the fresh and dried soil moisture content were strongly affected by the applied treatments, with B+ consistently showing lower moisture retention as compared to other treatments. This is in line with other studies using the Tukey HSD test, where significant differences in soil moisture have been detected between certain irrigation or management treatments, indicating that soil amendments, management, or even specific plant-microbe interventions can create measurable differences in soil water retention capacity. Studies have repeatedly showed that soil moisture responds sharply to both biological treatments and physical processes like drying and rewetting. Similar to the present results, Emran et al. (2024), significant moisture differences using Tukey HSD tests among irrigation treatments, leading to variable carbon and nutrient cycling responses. This aligns with the pronounced differences between B+ and B- in dataset. Further, Ricks and Yannarell (2023), and Collings et al. (2025), document that soil moisture modulates the activity and competitive effects of soil microbes, which in turn alters plant community feedbacks and soil nutrient dynamics, potentially explaining why some treatments retain less water than others. The drying-rewetting process itself is known to cause biogeochemical changes in soils, disrupting microbial biomass and altering nutrient availability and carbon release. This is relevant for interpreting why the relative ranking of the treatments did not shift drastically after drying, physical changes in water content may reinforce existing differences established by biotic or treatment effects. The consistently lower moisture under B+ treatment could indicate heightened microbial decomposition, altered soil structure, or enhanced nutrient cycling tied to the specific treatment. In literature, high soil microbial activity and certain plant-microbe manipulations have been shown to accelerate water loss or soil organic matter turnover, sometimes leading to lower soil moisture content. The visual confidence plots confirm these statistical results, highlighting that only pairwise treatment comparisons involving B+ versus B- or A- reach the threshold of significance. This underscores the robustness of the detected differences and matches documented expectations from similar agricultural and ecological studies. These findings contribute to the broader understanding that soil management strategies can dramatically affect soil moisture dynamics even after a drying cycle. This is especially relevant in agroecological contexts, where soil moisture regulation is key to improving plant growth, crop yield, and resilience to drought. The literature supports that such changes in moisture also show into plant performance, nutrient cycling, and longer-term soil health, suggesting that selecting soil management/regeneration strategies should account for their impact on soil water dynamics. In summary, the results align with broader literature results showing that targeted treatments can cause pronounced, statistically significant variations in soil moisture. These differences are persistent through drying cycles and have important implications for soil-microbe-plant interactions and agroecosystem management.

Soil infiltration rate (*Figure 28*) was measured both before and after planting in the different treatments, yet no significant differences emerged among the groups at either time point. This pattern highlights that, under the given conditions, neither tillage practice nor microbial inoculation, nor the transition from pre-planting to post-establishment, led to measurable changes in infiltration. In both pre-planting and post-planting measurements, mean infiltration rates were similar across the four treatments, with overlapping error bars and family-wise confidence intervals. This suggests a degree of stability in soil structure or water movement properties regardless of treatment or crop establishment. Literature shows that the effect of planting (crop establishment) on infiltration is often variable. Some studies indicate that as plant roots develop, they can increase infiltration by improving soil structure and pore connectivity. However, in managed agricultural systems, these changes may require longer periods or more intense biological activity to be measurable (Leung et al., 2017; Zhu et al., 2020). Studies have shown that infiltration can either improve or remain unchanged after planting, depending on factors like soil type, cropping system, and management duration. For example, Leung et al. (2017), found a linear increase in infiltration rate with plant age and root development, but these effects were strong on soils with more responsive structures and under long-term vegetation. Infiltration rates sometimes remain stable after planting when initial soil conditions (such as compaction or aggregate stability) dominate water movement, and when root growth or microbial activity are not sufficient to alter these properties within the timescale of the study (Söderberg, 2015). The lack of observable change before and after planting in this study suggests that under the tested management conditions, or over the experimental duration, root or microbial effects on infiltration were modest. Root growth, while known to increase soil porosity in some contexts, may not have reached a threshold to produce measurable differences. Literature reviews conclude that the greatest and most consistent improvements in infiltration follow sustained use of cover crops, organic amendments, or multi-season conservation practices, rather than single interventions or short trials (Thierfelder and Wall, 2009). The results here are consistent with such findings, supporting the idea that single-season or modest biological interventions may not always yield significant immediate impacts on soil infiltration dynamics (Castiglioni et al., 2018). In summary, infiltration rate did not differ significantly between treatments or from before to after planting, which is well-aligned with existing literature showing that observable changes in infiltration may require longer-term interventions or more transformative management practices.

3.5. Shotgun Metagenomic Sequencing of Soil Microbiomes

Three metagenomic soil samples from the same garden were subjected to shotgun sequencing and comparative analysis to profile their microbial communities. The results illuminate the diversity and complexity typical of environmental soil microbiomes.

Overall Classification and Read Distribution

A substantial proportion of the reads in each dataset, from approximately 93.8% to 94.9%, could not be classified at high taxonomic levels. This observation is consistent with common outcomes in environmental metagenomics and reflects the prevalence of uncharacterized or novel microbial sequences. Among the classified fraction, bacteria represented 4.8% to 5.8% of the total reads and displayed rich taxonomic diversity.

Dominant Bacterial Phyla

Actinobacteria: Dominated the classified reads, varying from 1.7% to 2.2% across samples. Within this phylum:

The *Streptomycetales* order, especially *Streptomyces,* was prominent.

Notable Streptomyces species detected included:

- *S. venezuelae*
- *S. griseus*
- *S. coelicolor*
- *S. avermitilis*
- *S. albus*
- *S. globisporus*
- *S. rimosus*
- *S. clavuligerus*
- *S. lividans*
- *S. scabiei*
- *S. cattleya*

- *S. hygroscopicus*
- *S. ambofaciens*
- *S. mobaraensis,* among many others.

In addition, substantial reads were assigned to other actinobacterial genera such as:

- *Kitasatospora (*including *K. setae)*
- *Streptacidiphilus*
- *Microbacterium (M. testaceum, M. foliorum)*
- *Agromyces (A. ramosus)*
- *Rathayibacter*
- *Curtobacterium (C. flaccumfaciens)*
- *Corynebacterium (C. glutamicum)*
- *Saccharopolyspora (S. erythraea, S. rectivirgula)*
- *Leucobacter*

Proteobacteria: Present at lower yet consistent abundance, heavily represented by **Alphaproteobacteria.**

Orders and genera observed included:

- ***Rhizobiales:***
- *Bradyrhizobiaceae (Bradyrhizobium japonicum, B. elkanii)*
- *Rhizobiaceae (Rhizobium leguminosarum, Agrobacterium tumefaciens)*
- *Methylobacteriaceae (Methylobacterium radiotolerans)*
- *Phyllobacteriaceae (Phyllobacterium brassicacearum)*
- *Sphingomonadaceae (Sphingomonas wittichii)*
- *Caulobacteraceae (Caulobacter crescentus)*

Other classes detected at lower abundance include B*etaproteobacteria* and *Gammaproteobacteria,* with genera such as *Burkholderia, Cupriavidus, Ralstonia,* ***Pseudomonas (P. putida, P. fluorescens),*** *Xanthomonas, Stenotrophomonas,* and *Acinetobacter.*

Firmicutes: Family *Bacillaceae* prominently represented:

- *Bacillus cereus*
- *B. thuringiensis*
- *B. anthracis*

- *B. mycoides*
- ***B. subtilis***
- *B. amyloliquefaciens*
- *B. licheniformis*

Other genera include *Paenibacillus (P. polymyxa), Staphylococcus (S. epidermidis, S. saprophyticus), Listeria,* and *Clostridium species.*

Other Phyla: Cyanobacteria: Detected at low levels. Genera included:

- *Synechococcus elongatus*
- *Nostoc punctiforme*
- *Anabaena variabilis*

Chloroflexi: Minor group, with representatives like *Caldilinea aerophila* and *Anaerolinea thermophila.*

The shotgun metagenomic data in your Excel file show that, in addition to bacteria, your soil samples contain diverse fungi, protists, archaea, plant sequences and animal-derived reads, which you can briefly highlight in the metagenomics chapter.

Other taxonomic groups beyond bacteria

In the non-bacterial fraction, fungi (kingdom Fungi) are well represented, including numerous Ascomycota (e.g. Sordariomycetes such as *Fusarium*, *Colletotrichum* and *Thermothielavioides*) and Basidiomycota (e.g. *Ustilago*, *Sporisorium*, *Cryptococcus*, *Malassezia*). These taxa include typical soil and plant-associated fungi, some of which comprise known plant pathogens (for example *Fusarium oxysporum*, *Colletotrichum higginsianum*, *Pyricularia* spp., *Botrytis cinerea*) and saprotrophs that may participate in organic matter decomposition.

Protist reads are present from several major lineages, including Apicomplexa (e.g. *Toxoplasma*, *Besnoitia*, *Plasmodium*, *Theileria*, *Babesia*), free-living amoeboid forms such as *Dictyostelium*, and flagellated groups like Kinetoplastea (*Leishmania*, *Trypanosoma*). While many reference genomes are parasitic or medical/veterinary model species, their detection in soil metagenomes usually reflects environmental relatives or low-level DNA traces rather than active infections in the soil community.

Archaeal diversity is also substantial, with Euryarchaeota (e.g. methanogenic lineages such as *Methanosaeta*, *Methanoculleus*, *Methanoregula*, *Methanocella*) and halophilic Halobacteria

(*Haloferax*, *Halorubrum*, *Halobacterium* and related genera) detected. These archaeal groups may indicate the presence of anaerobic microsites where methanogenesis can occur and, depending on site conditions, niches with elevated salinity favouring halophiles.

Plant-derived sequences form a conspicuous portion of the non-bacterial reads, including many embryophyte and crop genomes such as *Vigna*, *Glycine*, *Phaseolus*, *Cicer*, *Medicago*, *Helianthus*, *Brassica*, *Zea mays*, *Sorghum bicolor*, *Oryza sativa* and *Beta vulgaris*, as well as woody plants like *Eucalyptus*, *Populus* and citrus species (e.g. *Citrus sinensis*). These reads likely originate from root and rhizosphere material, decaying plant residues and chloroplast/mitochondrial genomes, and they can be used to support your description of the plant community and cropping history of the sampled plots.

Finally, a smaller but notable fraction of reads is assigned to Metazoa, including chordates such as *Homo sapiens*. These assignments represent animal and human DNA contamination (e.g. skin cells, small vertebrate activity, laboratory or handling contamination) rather than members of the soil microbiome sensu strictu, and they support a short discussion of unavoidable background DNA in shotgun datasets.

Taxonomic limitations and contamination

Although shotgun metagenomics allows taxonomic assignment down to the putative species level, species-level identifications must be interpreted with caution because they rely on similarity to available reference genomes and can be affected by conserved regions, incomplete databases and misannotations. This is particularly relevant for groups where the reference set is dominated by model organisms or pathogens (e.g. human, crop and clinical strains), meaning that hits to named species often represent the closest sequenced relative rather than the true environmental species present in the soil. In addition, the dataset contains a non-negligible number of reads assigned to plant genomes (including crop and tree species) and metazoans such as *Homo sapiens*, which most likely reflect chloroplast or mitochondrial sequences, root and litter DNA, or low-level animal and human contamination introduced during sampling and laboratory processing. These potential contaminants were not the focus of the present ecological analysis, but their presence underlines the importance of careful data filtering and of distinguishing genuine soil microbiome members from background DNA when interpreting metagenomic profiles.

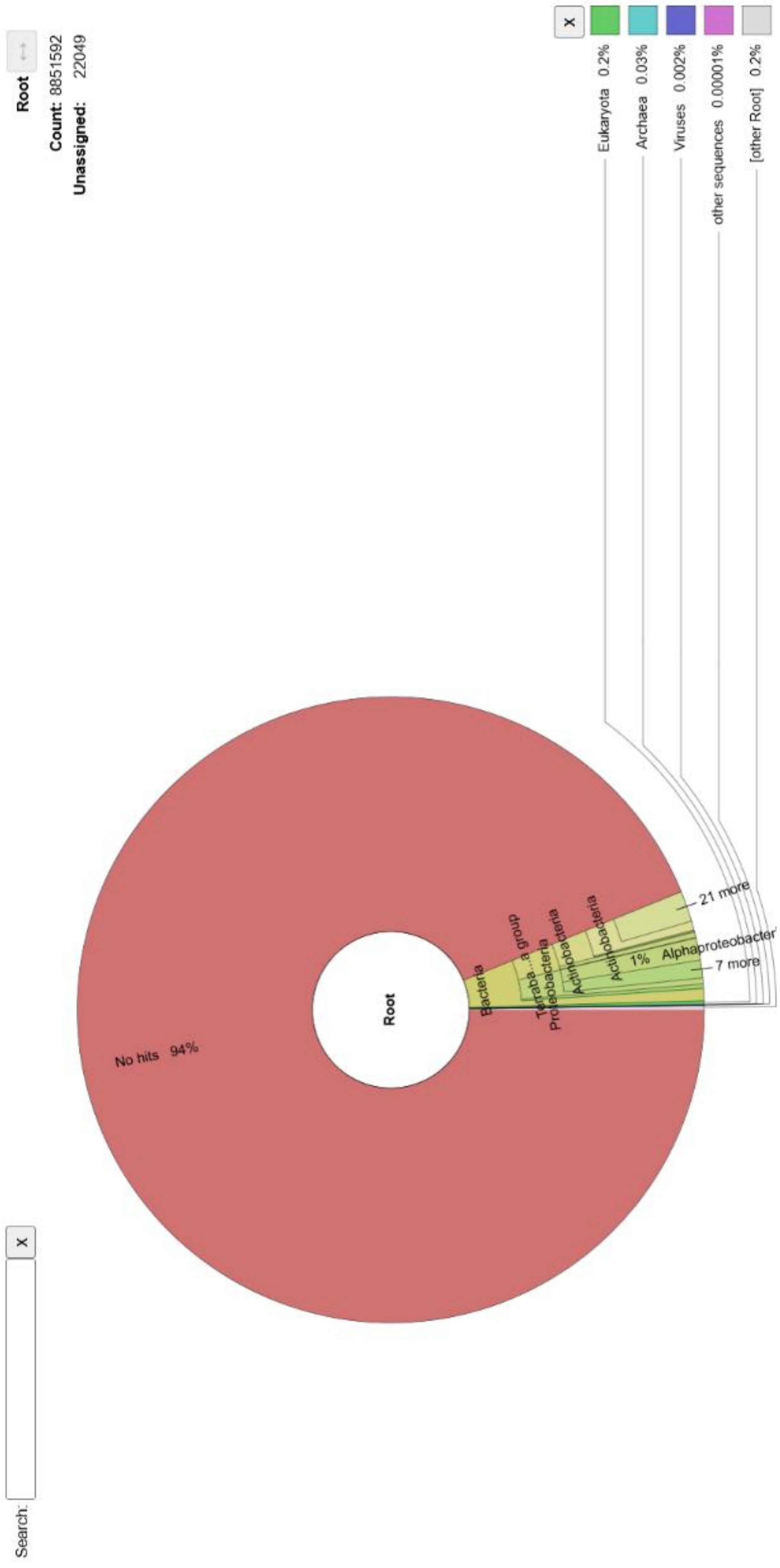
Diversity Patterns and Comparative Notes

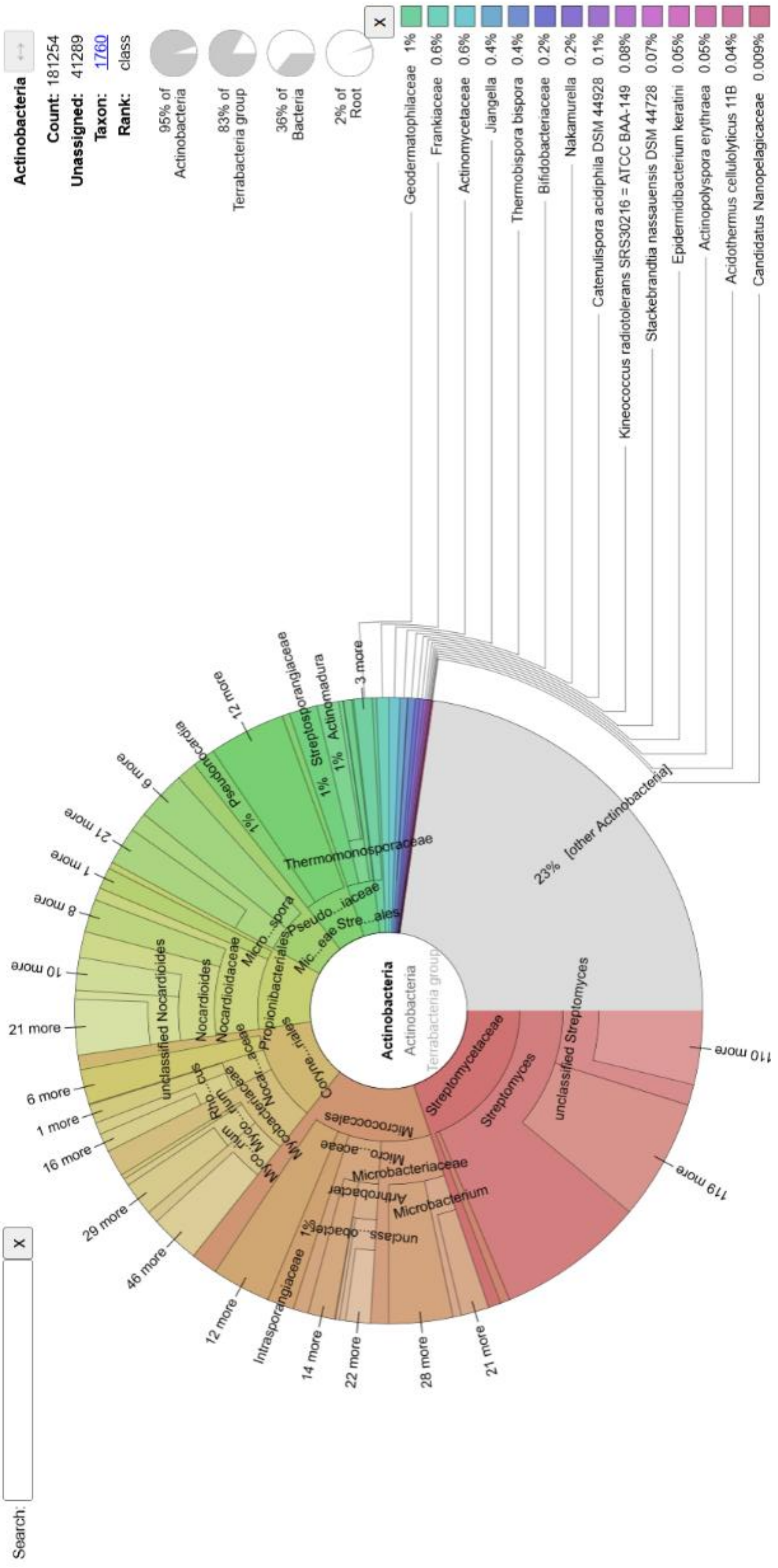
Taxonomic profiles were mostly consistent between SZIA_garden_1 and SZIA_garden_2, both showing similar dominance and diversity within key actinobacterial and proteobacterial groups. SZIA_garden_3 exhibited a slightly reduced proportion of total classified bacterial reads and Actinobacteria but otherwise showed comparable taxonomic structure and species richness. Some genera and species exhibited minor variations in abundance, possibly reflecting microhabitat differences.

Collectively, the three metagenomes from garden soil reveal a diverse, Actinobacteria- and Proteobacteria-rich microbiome, underpinned by a vast reservoir of as-yet unclassified bacteria. The detectable richness at the genus and species levels, particularly among actinobacterial taxa, highlights the soil's healthy, active, and complex microbial ecosystem. These findings affirm the central role of soil bacteria in maintaining ecosystem function and underline the unexplored potential within unclassified read sequences.

On *Figure 29, 30* and *31* are shown Krona Charts of all 3 samples.

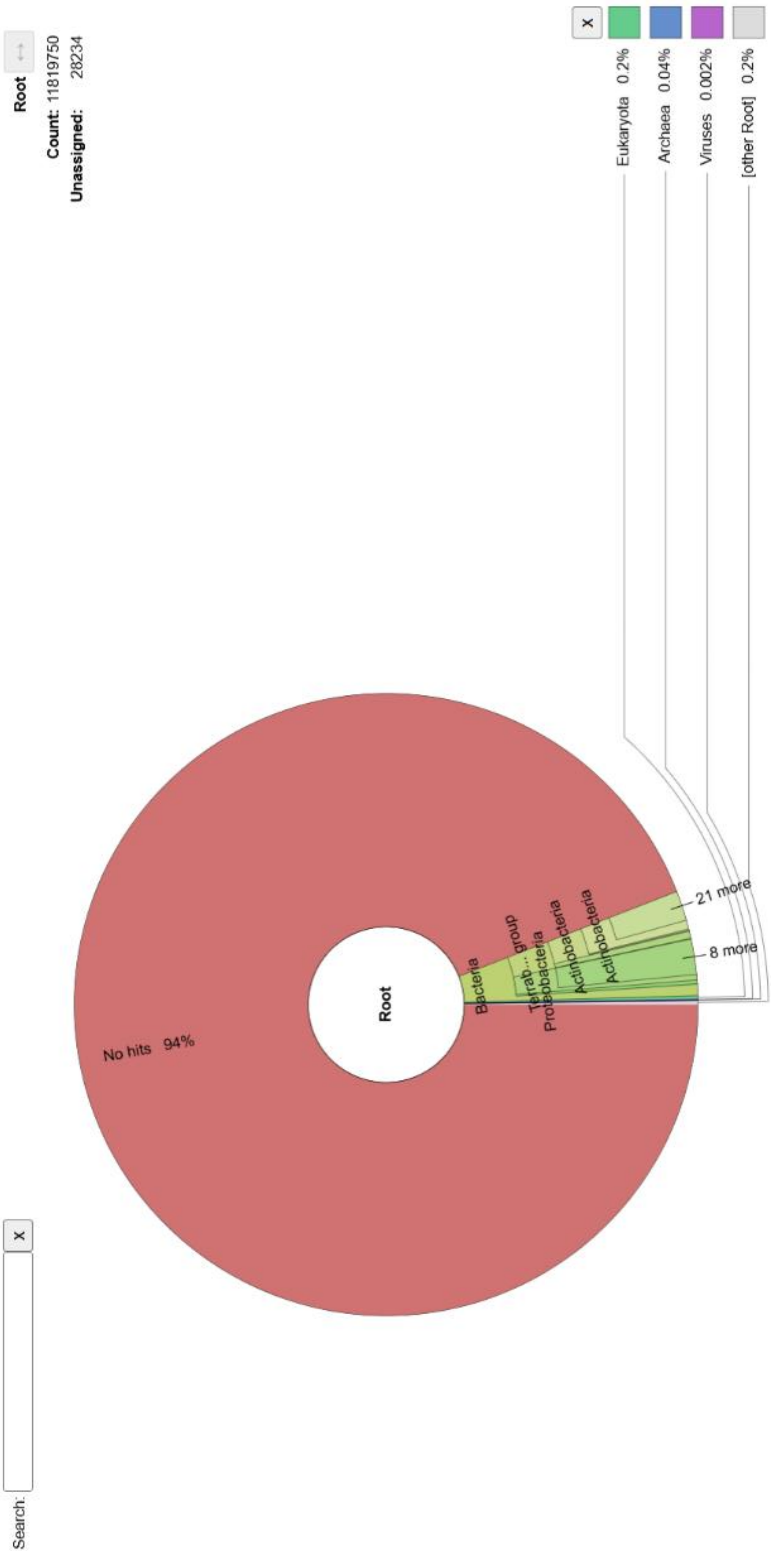
Figure 29: Krona Chart of SZIA 1 Sample_Zoom 1 and Zoom 2

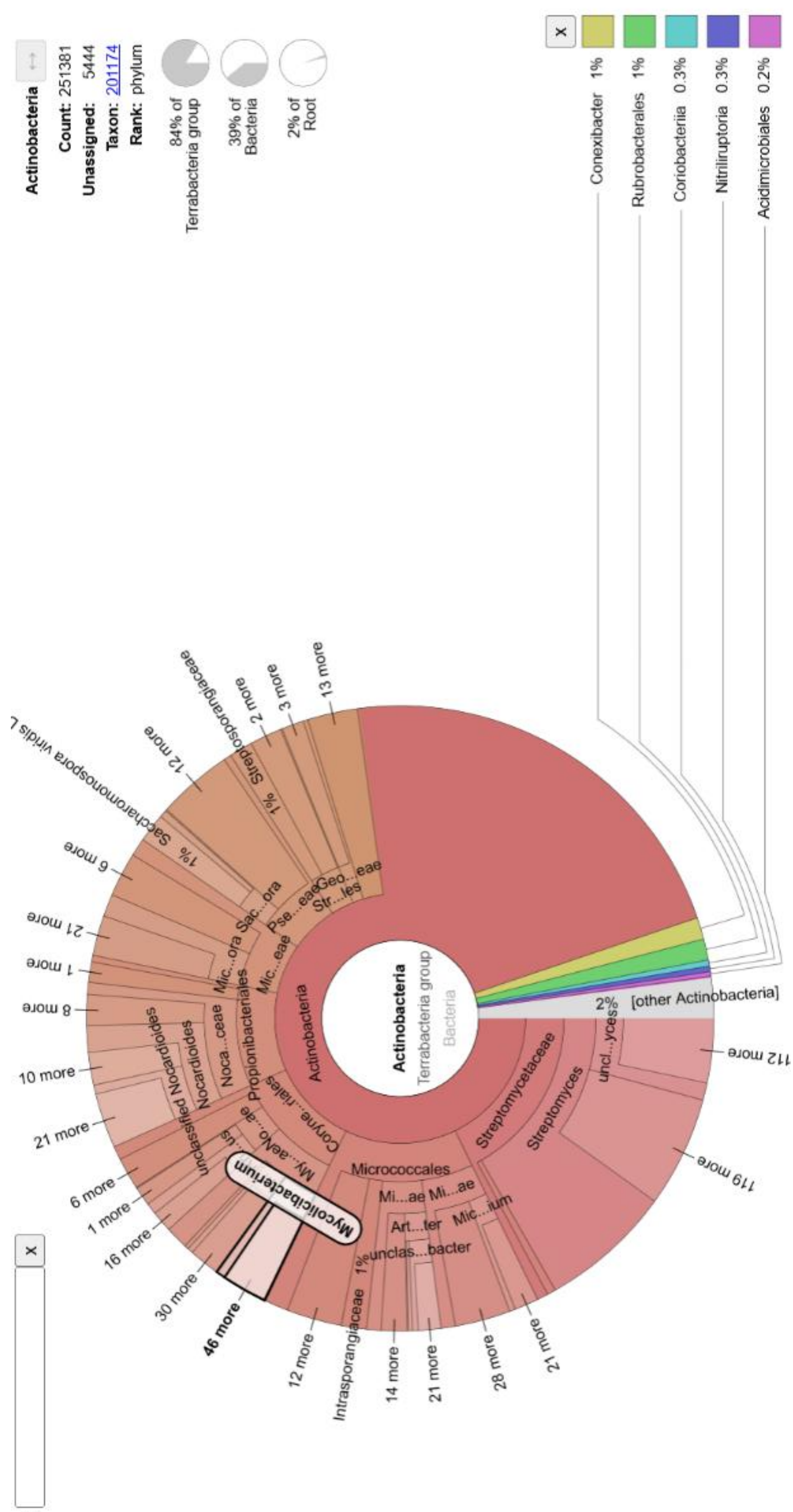




Source: Chart provided by the laboratory

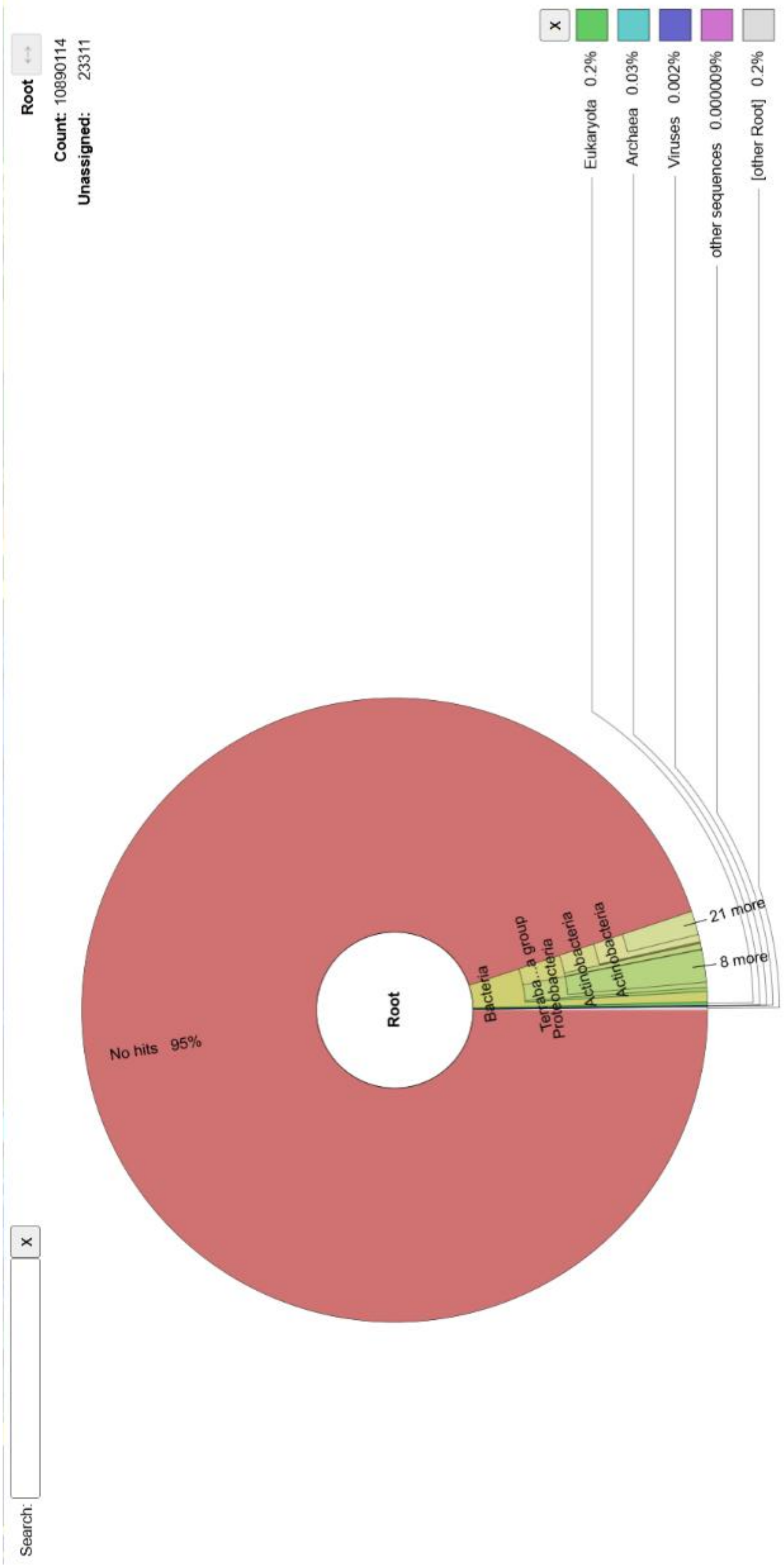
Figure 30: Krona Chart of SZIA 2 Sample_Zoom 1 and Zoom 2

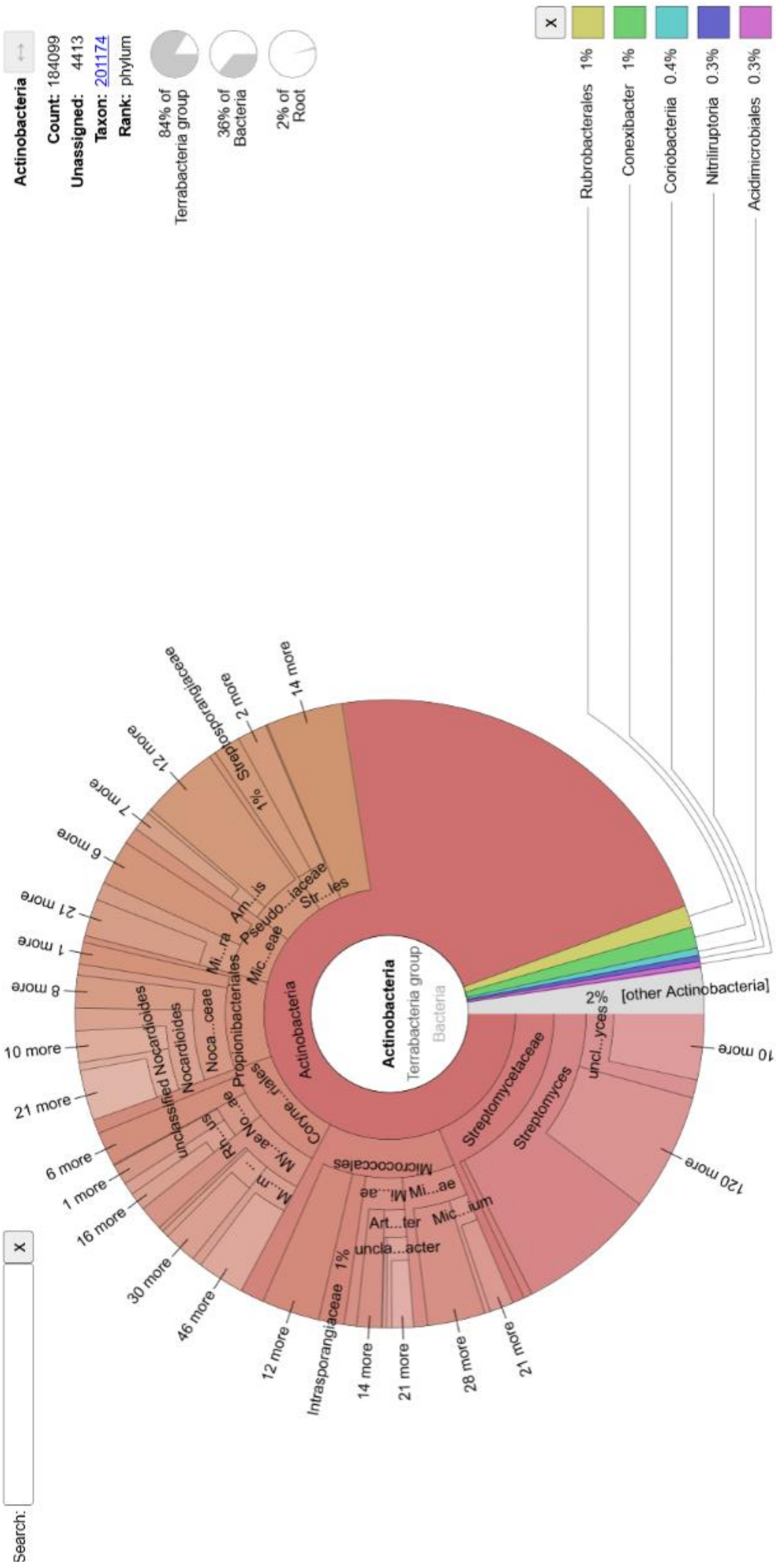




Source: Chart provided by the laboratory

Figure 31: Krona Chart of SZIA 3 Sample_Zoom 1 and Zoom 2





Source: Chart provided by the laboratory

Analysing the description of the area, the examination of metagenomic analysis from soil samples reveals the diversity and complexity of microbial life found within garden soil microbiomes. This finding aligns with previous research that recognizes soil as one of the most diverse microbial environments on the planet. Notably, the fact that a significant portion of reads (93.8%-94.9%) remains unclassified supports existing literature on the abundance of uncultured and novel microbial taxa in soil metagenomes, highlighting the extensive unexplored "microbial dark matter" (Delmont et al., 2011).

Actinobacteria dominated the classified bacterial reads, a pattern in line with their established ecological prominence in soil as major decomposers of complex organic compounds and contributors to carbon cycling. The predominance of *Streptomyces* species, a genus well-known for their filamentous growth, production of extracellular enzymes, and biocontrol properties, reinforces their role in maintaining soil health and nutrient cycling. The detection of many *Streptomyces* and *Kitasatospora* species underscores the soil's capacity for organic matter turnover and biotic interactions. Other actinobacterial genera detected, such as *Microbacterium* and *Corynebacterium*, further emphasize the metabolic diversity and functional redundancy typical of soil actinobacterial communities (Bhatti et al., 2017).

Proteobacteria, particularly *Alphaproteobacteria*, were consistently present, reflecting their functional versatility in soils, including nitrogen fixation, organic matter degradation, and interactions with plant roots. Genera such as *Bradyrhizobium, Rhizobium,* and *Agrobacterium* are notable for their symbiotic and plant-associated lifestyles, which play critical roles in nitrogen cycling and plant health promotion (Vieira et al., 2021). *Bacillus* species are acknowledged for their capacity to degrade soil organic matter and contribute to nutrient cycling, as well as producing compounds that protect plants and enhance soil structure (Saxena et al., 2020).

This research primarily focused on bacterial inoculants including *Pseudomonas spp., Azotobacter spp., Bacillus spp., Rhizobium spp.,* and *Ensifer spp.* These species were also detected as components of the soil microbial community.

In summary, the metagenomes underscore a diverse, *Actinobacteria* and *Proteobacteria*-rich soil microbiome with substantial unexplored genetic potential. The dominance of *Streptomyces* and other key actinobacterial taxa highlights their indispensable roles in organic matter decomposition and soil health maintenance. *Proteobacteria* complement this with nutrient cycling and plant symbioses, while *Firmicutes* and minor phyla contribute to ecosystem multifunctionality (Nesme et al., 2016).

This soil microbial community supports ecosystem stability, nutrient cycling, and plant productivity, emphasizing the critical importance of characterizing unclassified sequences to unlock soil biodiversity's full potential. These findings align with broad metagenomic and ecological studies emphasizing soil as a reservoir of microbial diversity essential for ecosystem functioning and sustainability.

4. CONCLUSION

This research evaluated the combined influence of conservation tillage (loosening, no-tillage) and microbial inoculation on soil quality and crop performance (potato (*Solanum tuberosum*), pea (*Pisum sativum*), lettuce (*Lactuca sativa*)) in sandy loam soil conditions characteristic of small-scale Hungarian agriculture.

Across the crop rotation, no statistically significant differences were detected in physical plant properties (plant size, biomass, tuber/root development). However, treatments combining microbial inoculation with reduced soil disturbance (loosening or no-tillage) consistently supported improved plant establishment and growth stability, reflecting enhanced soil-plant-microbe interactions that may require longer periods to result in measurable physiological differences.

Regarding the chemical properties of plants, all measured nutrient and biochemical parameters fell within the ranges reported in scientific literature, confirming optimal plant physiological functioning. One statistically significant difference was found in peas, where total sugar content varied between treatments. Peas grown under loosening and microbial inoculation showed a decrease in sugar content compared to the no-till, no inoculation. This suggests that microbial activity may influence carbon allocation in developing seeds, possibly shifting resources from simple sugar accumulation toward protein metabolism.

For soil parameters, significant differences were detected in the first year in soil plasticity (Arany value) and pH(KCl) between inoculated and non-inoculated soils, proving that microbial inoculants can alter soil structure and chemical conditions even within one growing season.

Across the measured soil physical properties, significant treatment effects were detected for soil penetration resistance and soil moisture, while infiltration rate showed no significant differences. Microbial inoculation consistently improved soil physical conditions: it reduced penetration resistance in both loosening and no-tillage systems, with the strongest effects observed in B+ (no-tillage with microbes), where resistance was significantly lower than in A+ (loosening with microbes) and B− (no-tillage without microbes). These results indicate that microbes can enhance soil structure and mitigate compaction, particularly under no-tillage. Soil moisture also differed significantly among treatments, with B+ (no-tillage with microbes) exhibiting consistently lower moisture content than B− (no-tillage without microbes) before drying and A- (loosening without microbes) after drying, confirming that management and microbial inputs measurably affected water retention. In contrast, infiltration rates remained statistically unchanged across all treatments, and between pre- and post-planting measurements, suggesting that short-term or single-season interventions were insufficient to alter infiltration dynamics. Overall, the significant results demonstrate that microbial amendments and conservation tillage practices can influence soil compaction and moisture dynamics, even when infiltration remains stable.

The absence of additional significant differences can be explained by the prior management history of the experimental area. Before the trial, the field had not been subjected to regular conventional ploughing and had remained fallow for a period, resulting in relatively undisturbed and moderately improved soil conditions at the outset. Under such circumstances, large contrasts among treatments are less likely to emerge within the first years of the experiment, as the baseline soil state was already favourable. Consequently, the effects of microbial inoculation were incremental rather than immediate. Soil regeneration is a gradual process, with early improvements often appearing as enhanced moisture regulation or subtle gains in soil structure before becoming statistically significant.

Referring to the *Hypothesis that conservation tillage improves soil structure and enhances plant physical properties,* the physical characteristics of plants, including growth, root development, and yield quality, did not show significant differences between treatments in the first years. However, plots with loosening and no-tillage consistently supported higher averages in plant establishment and growth. Reduced tillage maintained higher soil moisture and lower soil resistance, creating more favorable rooting conditions. These observations indicate that soil structure was already beginning to respond positively to reduced disturbance, supporting the Hypothesis 1 even though yield differences were not yet statistically measurable.

Referring to *Hypothesis that Microbial inoculation enhances plant biochemical properties and improves crop quality,* for plant chemical parameters, all crops remained within the expected physiological ranges reported in the literature, confirming the stability of the production system. A statistically significant difference appeared in total sugar content of peas, where the no-tillage, no microbes showed the highest sugar concentration and the loosening + microbes treatment resulted in lower sugar content. This outcome aligns with findings from literature demonstrating that soluble sugars in peas are highly sensitive to environmental and management conditions. A possible explanation is that microbial inoculation stimulates root nutrient uptake, especially nitrogen, redirecting carbon away from sugar accumulation and toward protein synthesis. Hypothesis 2 is partially supported: Microbial inoculation influenced plant biochemistry, but not always in the expected direction.

Referring to *Hypothesis that The combination of conservation tillage and microbial inoculation results in synergistic benefits for soil and crop performance,* for soil parameters, statistically significant treatment effects appeared in: soil plasticity (Arany) and soil pH(KCl) after first year of research, with these changes occurred after only one year, indicating that microbial inoculation can initiate measurable shifts in soil behaviour. Soil moisture and resistance also improved under reduced tillage, particularly when inoculation was combined with loosening or no-tillage. In the second and third year, soil parameters remained within agronomically acceptable ranges, but differences between treatments were no longer statistically significant, most likely because the site had already been managed under agroecological principles before this study, giving it a head start in soil regeneration. Hypothesis 3 is supported: The combination treatment consistently performed well, even without a strong first-year statistical separation.

Thus, the research bridges the gap between short-term agronomic observations and long-term soil regeneration, contributing unique data on how microbial inoculants function as part of an integrated agroecological system.

While short-term measurable differences were limited, the study clearly demonstrated that:

- Microbial inoculants are safe, compatible with conservation tillage, and may influence plant biochemical pathways.
- Conservation tillage improves soil moisture retention and lowers compaction, critical functions in sandy soils.
- Combined treatments produced the most favourable results (in terms of highest averages) and are likely to improve further with ongoing application.
- Soil and plant responses in regenerative systems are gradual and cumulative, and sandy soils particularly require time to show measurable improvement. The observations indicate that the system is moving in a regenerative direction, and continued monitoring will likely reveal more pronounced differences as soil structure, organic matter, and microbial communities become increasingly stable.

In summary, this research demonstrates that integrating microbial inoculation with reduced tillage is a promising approach for improving plant performance and soil quality, supporting the transition toward agroecological and climate-resilient farming systems.

5. NOVELTY OF THE RESEARCH AND NEW SCIENTIFIC FINDINGS

The novelty of this research lies in its integration of multiple sustainability-oriented agronomic dimensions that are rarely examined simultaneously within a single field experiment:

- **Combined evaluation of microbial inoculation, conservation tillage intensity (loosening vs. no-tillage), and a market-garden crop rotation (potato-pea-lettuce),** a rotation specifically designed for small-scale agroecological vegetable systems.
- **Simultaneous assessment of plant performance (physical and chemical traits) and soil quality (physical, chemical, and biological indicators)** across three consecutive cropping seasons under identical field conditions.
- **Three years monitoring,** allowing the cumulative and time-dependent effects of microbial inoculation to be captured, an aspect often missing in studies limited to single-season trials.
- **Holistic soil assessment,** including penetration resistance, moisture dynamics, infiltration, chemical fertility parameters, and biological indicators, far beyond the single scope (e.g., pH or SOM only) commonly found in existing publications.
- **Comprehensive plant analysis,** assessing multiple traits (e.g., nutrient content, biochemical) each year, whereas many existing studies examine only a single parameter such as vitamin C or chlorophyll.
- **High replicability,** as the experimental structure can be easily scaled to larger fields by proportionally adjusting microbial inoculant doses according to manufacturer recommendations.

The study provides several new scientific contributions. This study has:

1. **Demonstrated that microbial inoculation produces measurable, positive improvements in soil physical properties,** including enhanced moisture retention, reduced penetration resistance, significant difference in soil plasticity by Arany value, in both loosening and no-tillage systems. These effects were most pronounced under no-tillage combined with microbial amendments, reflecting early regenerative changes in soil structure that create favorable rooting conditions. These soil improvements supported better plant establishment, growth stability, and early indications of enhanced nutrient uptake, highlighting the integrated benefits of conservation tillage and microbial inoculation.

2. **Identified significant differences in soil moisture dynamics,** particularly between no-tillage with microbes and no-tillage without microbes, revealing strong treatment-dependent effects on water retention. **Confirmed that short-term interventions did not significantly affect infiltration rate,** supporting the hypothesis that infiltration improvements require longer-term or more transformative soil management practices.
3. **Provided a comprehensive, multi-year dataset integrating soil physical, chemical, and biological indicators** with plant morphological, physiological, and biochemical traits, representing one of the most detailed field-level assessments of microbial inoculant effects. This integrated dataset captures soil-plant-microbe interactions, early indicators of soil regeneration, and the influence of microbial amendments on plant establishment, growth stability, nutrient accumulation, and key quality parameters, including a significant increase in total sugar content in peas under inoculated treatments.
4. **Addressed an important knowledge gap,** by integrating microbial inoculation, conservation tillage, and market garden vegetable rotation within a controlled field experiment, an approach that has been rarely examined in published research while evaluating both soil and plant responses.

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https://www.researchgate.net/profile/Jana-Marjanovic-3

https://m2.mtmt.hu/gui2/?type=authors&mode=browse&sel=10095136

AUTOR’S BIOGRAPHY

Jana Budimir-Marjanović (née Marjanović) was born on February 7, 1998, in Berane, Montenegro. She completed her primary and secondary education in her hometown Berane, where she graduated with the honorary distinction *Luča* an award given to students who achieve excellent academic results throughout every year of their schooling.

Jana earned her Bachelor of Science degree in Food Technology, Food Safety, and Ecology at the University of Donja Gorica in 2019. During her undergraduate studies (2016-2019), she took an active part in international academic mobility, completing exchanges in Estonia and Hungary, as well as participating in short academic programs in Germany and China. These experiences broadened her worldview and sparked her long-term interest in sustainability and international scientific cooperation.

Following her bachelor studies, in 2020, Jana was awarded the *Stipendium Hungaricum* scholarship, one of Hungary’s most competitive academic scholarships, which enabled her to pursue her Master of Science degree in Environmental Sciences at Eötvös Loránd University (ELTE) in Budapest, Hungary. Her master’s research strengthened her focus on environmental sustainability, agroecology and supported her transition toward research and academia.

After completing her Master’s degree, in 2022, Jana received the *Stipendium Hungaricum* scholarship once again, this time to continue her education at the doctoral level, a four-year PhD program at the Doctoral School of Natural Sciences (Environmental Science Program) at the Magyar Agrár- és Élettudományi Egyetem (Hungarian University of Agriculture and Life Sciences-MATE) in Gödöllő, Hungary.

Her doctoral research focuses on agroecology, sustainable crop production and soil health, with particular emphasis on the use of beneficial soil microorganisms to strengthen agricultural resilience. By integrating scientific innovation with practical agroecological approaches, her work aims to contribute to the development of environmentally responsible agricultural practices.

Throughout her doctoral studies, Jana has actively participated in international scientific conferences and workshops across Europe and the United States, where she presented her research findings and collaborated with researchers and industry partners. She has authored and co-authored scientific publications in her research field.

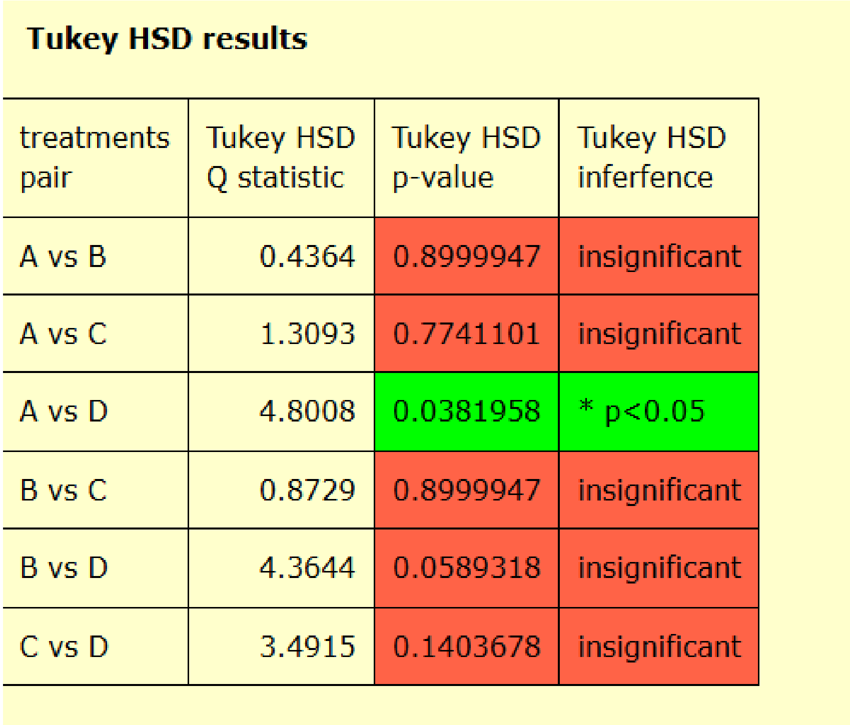
In recognition of her academic achievements and research potential, Jana received the *EIT Food RIS Fellowship in 2024 and 2025,* awarded by the European Institute of Innovation and Technology (EIT Food). This fellowship supports young researchers and enables collaboration with companies engaged in agricultural innovation.

Since 2021, Jana has been also involved in the energy consultancy field.

Jana is dedicated to advancing sustainable agriculture and agroecology, with a strong focus on environmentally responsible food production. Her educational journey reflects determination, curiosity, and a commitment to applying scientific research for the benefit of both society and the environment.

APPENDICES

Figure 32: Results of Tukey HSD post-hoc test for Total Sugar Content in Peas (II year-2024)



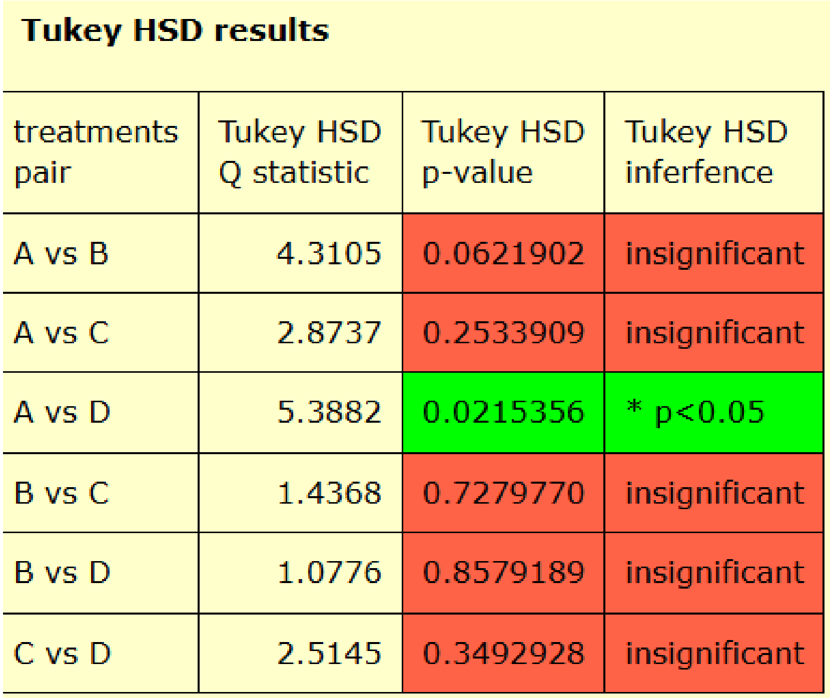
Tukey HSD results

treatments pair	Tukey HSD Q statistic	Tukey HSD p-value	Tukey HSD inferfence
A vs B	0.4364	0.8999947	insignificant
A vs C	1.3093	0.7741101	insignificant
A vs D	4.8008	0.0381958	* p<0.05
B vs C	0.8729	0.8999947	insignificant
B vs D	4.3644	0.0589318	insignificant
C vs D	3.4915	0.1403678	insignificant

Letters indicate statistically homogeneous groups (p < 0.05). Treatments compared: A+ (A), A− (B), B+ (C), and B− (D)

Source: Own work

Figure 33: Results of Tukey HSD post-hoc test for Soil Plasticity according to Arany in soil after Potato (I year-2023)



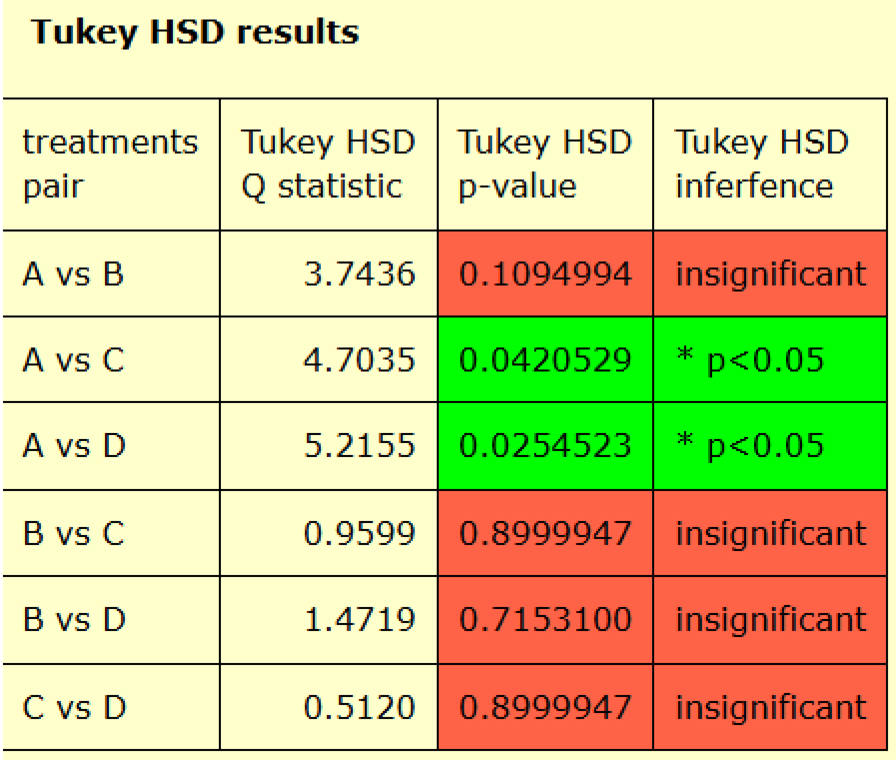
Tukey HSD results

treatments pair	Tukey HSD Q statistic	Tukey HSD p-value	Tukey HSD inferfence
A vs B	4.3105	0.0621902	insignificant
A vs C	2.8737	0.2533909	insignificant
A vs D	5.3882	0.0215356	* p<0.05
B vs C	1.4368	0.7279770	insignificant
B vs D	1.0776	0.8579189	insignificant
C vs D	2.5145	0.3492928	insignificant

Letters indicate statistically homogeneous groups (p < 0.05). Treatments compared: A+ (A), A− (B), B+ (C), and B− (D)

Source: Own work

Figure 34: Results of Tukey HSD post-hoc test for Soil pH(KCl) in soil after Potato (I year-2023)



Tukey HSD results

treatments pair	Tukey HSD Q statistic	Tukey HSD p-value	Tukey HSD inferfence
A vs B	3.7436	0.1094994	insignificant
A vs C	4.7035	0.0420529	* p<0.05
A vs D	5.2155	0.0254523	* p<0.05
B vs C	0.9599	0.8999947	insignificant
B vs D	1.4719	0.7153100	insignificant
C vs D	0.5120	0.8999947	insignificant

Letters indicate statistically homogeneous groups (p < 0.05). Treatments compared: A+ (A), A− (B), B+ (C), and B− (D)

Source: Own work

Table 20: Tukey HSD Pairwise Comparisons of Soil Penetration Resistance Before Planting (Peas II year-2024)

Comparison	Difference in Means	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound	Adjusted p-value	Significance
A+ - A-	-0.34116	-0.78766	0.10534	0.19753	Not significant
B- - A-	-0.07768	-0.52418	0.36882	0.96896	Not significant
B+ - A-	-0.60533	-1.05183	-0.15883	0.00322	**Significant**
B- - A+	0.26348	-0.18302	0.70998	0.41910	Not significant
B+ - A+	-0.26417	-0.71067	0.18233	0.41674	Not significant
B+ - B-	-0.52765	-0.97414	-0.08115	0.01351	**Significant**

Source: Own work

Table 21: Tukey HSD Pairwise Comparisons of Soil Penetration Resistance During Planting (Peas II year-2024)

Comparison	Difference in Means	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound	Adjusted p-value	Significance
A+ - A-	-0.4304	-0.8524	-0.0083	0.0438	**Significant**
B- - A-	-0.0625	-0.4846	0.3596	0.9804	Not significant
B+ - A-	-0.4446	-0.8667	-0.0226	0.0347	**Significant**
B- - A+	0.3679	-0.0542	0.7896	0.1108	Not significant
B+ - A+	-0.0143	-0.4363	0.4077	0.9998	Not significant
B+ - B-	-0.3821	-0.8041	0.0399	0.0909	Not significant

Source: Own work

Table 22: Tukey HSD Pairwise Comparisons of Soil Penetration Resistance After Planting (Peas II year-2024)

Comparison	Difference in Means	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound	Adjusted p-value	Significance
A+ - A-	-0.2502	-0.6757	0.1754	0.4226	Not significant
B- - A-	0.2954	-0.1302	0.7210	0.2749	Not significant
B+ - A-	-0.0690	-0.4945	0.3566	0.9747	Not significant
B- - A+	0.5456	0.1200	0.9712	0.0060	**Significant**
B+ - A+	0.1812	-0.2444	0.6068	0.6850	Not significant
B+ - B-	-0.3644	-0.7899	0.0612	0.1210	Not significant

Source: Own work

Table 23: Tukey HSD Pairwise Comparisons of Soil Moisture for Fresh Samples (Before Drying) (Peas II year-2024)

Comparison	Difference in Means	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound	Adjusted p-value	Significance
A+ - A-	-1.2004	-9.9524	7.5516	0.9822	Not significant
B- - A-	3.3131	-5.4389	12.0651	0.7358	Not significant
B+ - A-	-6.2835	-15.0355	2.4685	0.2299	Not significant
B- - A+	4.5135	-4.2385	13.2656	0.5102	Not significant
B+ - A+	-5.0831	-13.8351	3.6689	0.4075	Not significant
B+ - B-	-9.5966	-18.3486	-0.8446	0.0272	**Significant**

Source: Own work

Table 24: Tukey HSD Pairwise Comparisons of Soil Moisture for Dry Samples (After Drying) (Peas II year-2024)

Comparison	Difference in Means	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound	Adjusted p-value	Significance
A+ − A−	-0.7327	-2.8560	1.3907	0.7864	Not significant
B− − A−	-1.6933	-3.8166	0.4301	0.1562	Not significant
B+ − A−	-2.6399	-4.7633	-0.5166	0.0102	**Significant**
B− − A+	-0.9606	-3.0839	1.1627	0.6155	Not significant
B+ − A+	-1.9073	-4.0307	0.2161	0.0909	Not significant
B+ − B−	-0.9467	-3.0701	1.1767	0.6264	Not significant

Source: Own work