Hungarian University of Agriculture and Life Sciences

ENHANCING SOIL FERTILITY AND CROP PRODUCTIVITY THROUGH DIVERSIFIED COVER CROPPING: A COMPREHENSIVE ANALYSIS OF GREEN MANURE EFFECTS IN CONTROLLED AND FIELD ENVIRONMENTS

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The PhD School

Name: MATE University

Field: Horticultural Sciences

Head: Prof. Dr. Éva Németh Zámboriné, DSc
Hungarian University of Agriculture and Life Sciences
Faculty of Horticulture
Department of Medicinal and Aromatic Plants

Supervisor: Prof. Dr. Borbála Biró, DSc
Hungarian University of Agriculture and Life Sciences
Institute of Environmental Sciences
Department of Agro-Environmental Studies

Co. Supervisor: Dr. Katalin Juhos, PhD
Hungarian University of Agriculture and Life Sciences
Institute of Environmental Sciences
Department of Agro-Environmental Studies

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

1. INTRODUCTION AND OBJECTIVES

The vitality and sustainability of agricultural systems hinge on the health of the soil, with cover crops playing a pivotal role in its enhancement (Snapp et al., 2005). The multifaceted impact of cover crops on soil properties and subsequent main crop biomass production has garnered significant attention in the realm of sustainable agriculture (Blanco-Canqui et al., 2015). This thesis explores the influence of different cover crop species and their management as green manure on soil characteristics, microbial activity, mycorrhizal colonization, and the performance of two main crops, corn and pepper, in diverse soil types across several three experimental settings. In a controlled greenhouse pot experiment, five distinct cover crop species were selected for their varied traits and potential soil health benefits. The cover crops were grown in pots filled with neutral pH sandy soil (Arenosols), a substrate chosen for its agricultural relevance and interactive potential with plant root systems. This meticulous setup aimed to minimize external variability, allowing for an accurate assessment of the cover crops' impact on soil quality and their biomass contribution as organic matter. Parallel to the pot experiment, a field study in Syria's Mediterranean climate introduced additional cover crop species suitable for inceptisol soils. This field experiment aimed to establish an initial understanding of the cover crops' influence on unaltered soil properties.

The synchronization of the field study with the controlled greenhouse experiment provided a broader perspective on the performance of cover crops across varying environmental conditions. Expanding upon these initial studies, a multi-year pot experiment evaluated the enduring effects of cover crops on different soil types and the subsequent productivity of corn and pepper. Three contrasting soil types—Arenosols, Chernozems, and Luvisols—offered a spectrum of physico-chemical contexts to assess the adaptability and cumulative effects of cover cropping (Brady & Weil, 2008). This phase also introduced a detailed examination of soil microbial activity through FDA hydrolysis and quantification of labile carbon via POXC analysis, offering a window into the biological dynamics underpinning soil fertility (Gregorich et al., 2003). These comprehensive studies were underpinned by rigorous analytical procedures.Biomass production was meticulously measured for both fresh and dry weights, providing a metric for the organic input from cover crops to the soil. The soil microbial activity and mycorrhizal colonization assays gave insights into the biological enhancement of soil health (Smith & Read, 2008), while the measurement of soil electrical conductivity and concentrations of nitrate and ammonium served as proxies for nutrient availability and soil fertility status (Di & Cameron, 2002). This integrated approach aims to consolidate the role of cover crops as a crucial component of sustainable agriculture, proposing management practices that could revitalize soil systems and enhance crop productivity. By analyzing the data gathered from these experiments, this thesis will offer comprehensive insights into the advantages and practical applications of cover crops, shaping recommendations for future agricultural strategies.

Scientific questions, hypotheses:

i. How do different cover crop species and mixtures affect soil fertility and crop productivity in both controlled and field environments? Are there significant differences in their performance based on their symbiotic relationships with beneficial microorganisms?

ii. To what extent do different soil types (Arenosols, Chernozems, Luvisols) influence the effectiveness of cover crops and their impact on soil health parameters?

iii. How do cover crops affect soil nutrient dynamics, particularly nitrogen availability (NH_4^+, NO_3^-, Nmin), in different experimental conditions and geographical locations?

iv. Can cover crops significantly enhance soil biological activity and organic matter content, as measured by FDA hydrolysis and POXC, especially in low-quality soils?

v. How does the application of different cover crops affect the biomass production and yield of subsequent main crops (pepper, maize, lentil) under various soil conditions?

Based on those scientific questions, the following objectives and hypotheses were set:

- To select specific and variable, the most common and most-appropriate cover crop-(CC)-species with different abilities to plant-microbe interrelations. Compare performance, growth and biomass-production and support selection among laboratory and field conditions.
- To evaluate the performance of the selected cover crops of using different soil types in two relevant countries (Syria and Hungary), with particular soil-characteristics and soil-quality to compare their potential plant-growth-abilities among different environmental conditions.
- To assess the impact of different cover crops on soil nutrient capacity with a main focus on the nitrogen dynamics. This parameter is reflected in NH_4^+, NO_3^-, and mineral nitrogen (Nmin) concentrations in used soil types and experimental conditions in both countries.
- To evaluate the influence of cover crop-derived green manure on some soil health indicators, specifically to permanganate oxidizable soil-carbon (POXC) parameter and microbial enzymatic activity measured through fluorescein diacetate (FDA) hydrolysis, as potential soil biological parameter.
- To examine the effects of cover crop management on the biomass (i.e. yield) production of subsequent main crops (pepper, corn and lentil plants,) as important crops in horti-, agri-cultural practices worldwide.

Hypotheses:

To guide the analysis of the experimental data, several hypotheses were formulated.

On the basis of literary data we assume, that…

H-1*: *...there will be significant pozitive differences among selected cover crops species and its mixtures on the bases of their interrelation with beneficial symbiont microorganisms (considering the double-, simple-, or non-symbiont crops).

H-2: *...the response of cover-crop-performance will be highly dependent on the particular characteristics of main, representative soil types (Arenosols, Chernozems, Luvisols) and its unique soil-health characteristics.*

H-3: *...soil nutrient (Nitrogen) status and capacity of studied soils (assesed as NH_4+, NO_3-,* *Nmin) will be affected by the different cover crops and mixtures in comparison with the control (without cover crops).*

H-4: *...soil biological and the symbiosis performance indicators (FDA hydrolysis, Myco%) with data about nutrient availability (POXC) will be highly dependent on the used soils and its characteristics, influenced by the different cover crops species and mixtures.*

H-5: *...biomass- and yield-production of subsequent main crops (pepper, maize and lentils) will be dependent, when grown in soils previously treated with different cover crops and or mixtures. Differences will be significant according on the used CC plant-species.*

2. MATERIALS AND METHODS

Pot Experiment

The pot experiment was conducted under controlled greenhouse conditions at the Faculty of Agricultural and Environmental Sciences, MATE University, Buda Campus, Budapest, Hungary. The experiment utilized Arenosols soil with a pH of 7.4, and each treatment was replicated to ensure statistical validity. Soil classified as sandy texture was collected from the Soroksár Botanical Garden. This soil was selected for its commonality in agricultural settings and its relevance to the cover crops chosen for this study. Its physical and chemical properties, particularly its texture and pH, were expected to interact with the root systems and exudates of the cover crops, potentially influencing the overall soil health and the crops' growth performance. To evaluate the effects of different cover crop species on soil properties and subsequent main crop performance. the pot experiment was conducted utilizing five different cover crop species were chosen for their diverse traits and potential benefits to soil quality: *Vicia faba* (broad bean), *Phacelia tanacetifolia* (phacelia), *Avena strigosa* (black oat), *Brassica carinata* (Ethiopian mustard), *Vicia benghalensis* (purple vetch).These species were chosen for their diverse traits and potential benefits to soil quality, specifically their roles in single or double symbiosis. Broad bean and purple vetch were selected for their ability to form double symbiosis (with rhizobia for nitrogen fixation and mycorrhizal fungi for phosphorus uptake). Phacelia and black oat were chosen as single symbiosis species (mycorrhizal fungi only), while Ethiopian mustard represented a non-symbiotic plant. Additionally, a mixed crop treatment incorporating all five species and a control with non-planted pots were included to ascertain the individual and combined effects of these crops on soil properties. The temperature was maintained at an average of 19°C during the daytime and 10°C at night to reflect common seasonal variations. Humidity was controlled at 52%, which is within the favorable range for the growth of these crops. The experimental period spanned eight weeks, throughout this period, plants were watered and maintained under these controlled conditions to minimize external variability and stress factors. The CC were grown in standard plastic pots, each with a volume capacity of 2000 grams.

Field Experiment

The field experiment was conducted at Tishreen University in Latakia city, Syria. The site is characterized by a Mediterranean climate with mild, wet winters and hot, humid summers. The growing season was from March to May. The field site featured inceptisol soil that had not been previously planted, with a pH range from 6.8 to 7.9. Daytime temperatures ranged from 18°C to 23°C, while nighttime temperatures dropped to a cooler 12°C to 15°C. These fluctuations are reflective of the diurnal temperature variations that can impact plant growth and soil processes. The humidity levels were maintained around 60-65%, providing a moderately moist environment for crop development. Five cover crops were used in this experiment: - *Trifolium* spp. (clover) - *Lens culinaris* (lentil) - *Brassica napus* (rapeseed) - *Raphanus sativus* (radish) - *Secale cereale* (rye). A mixed treatment comprising all five species and control plots without any plant cover were also included. The experiment was arranged in a randomized block design with adequate replication with 4 blocks. Each plot measured 2*2 meters. The seeding rate for each cover crop species was approximately 50-100 seeds per designated plot, ensuring even distribution and emulating natural planting conditions. The plant density for each species was thus approximately 12.5-25 plants/m².

Examination of soil characteristics

Three soil types were selected for this study: **Arenosols**, **Chernozems**, **Luvisols**. Each soil type exhibits unique physico-chemical properties that influence the performance of cover crops and subsequent main crops.

Table 1. Physico-chemical characteristics of three soil types used in the experiment.

Soil type by WRB	**Texture**	**pH**	**SOM (%)**	**Bulk density (g/cm)**	**Water holding capacity (%)**	**Origin**
Arenosols	Sand	7.46	1.16	1.65	15	Soroksár Botanical Garden
Chernozems	Loam	7.35	2.81	1.35	40	Kömlőd (Komárom-Esztergom County)
Luvisols	Clay loam	4.91	1.64	1.25	50	Baranya County

Biological Properties Analysis: Fluorescein Diacetate (FDA) Hydrolysis Assay: FDA hydrolysis is an indicator of overall microbial activity in soil. Since microbes play a pivotal role in organic matter decomposition and nutrient cycling, high FDA activity suggests a vibrant microbial community and healthy soil. This parameter is used to assess the impact of agricultural practices on soil life (Schnürer & Rosswall, 1982; Biró et al., 2012). The procedure involved: 1. Placing 1 gram of soil into a test tube with 7.5 ml of 60 mM phosphate buffer 2. Adding 100 µl of FDA stock solution (2 mg/mL) 3. Incubating at 30. for 3 hours in a shaker incubator set at 200 rpm 4. Adding 7.5 ml of acetone to halt the reaction 5. Measuring fluorescence intensity using a spectrophotometer at 490 nm.Mycorrhizal Colonization Assessment: This assessment evaluated the presence and extent of arbuscular mycorrhiza fungi (AMF) colonization within the root systems of various plants. The methodology involved: 1. Washing plant roots using tap water to remove soil and debris 2. Softening roots in 7% potassium hydroxide (KOH) for 24 hours 3. Acidifying by soaking in 5% lactic acid 4. Staining using 0.01% aniline blue solution in 5% lactic acid for 24 hours 5. Examining thirty 1-cm root fragments microscopically 6. Quantifying colonization as MYCO% (percentage of root length colonized by AMF). Soil Chemical Parameters: Permanganate Oxidizable Carbon (POXC) Analysis: This analysis was employed to quantify the labile carbon fraction within the soil, which is readily oxidizable by potassium permanganate. This measurement serves as a critical indicator of soil health and fertility, given that labile carbon is pivotal for soil microbial activity and nutrient cycling. Electrical Conductivity (EC): EC measures soil salinity, indicating the concentration of soluble salts. High EC levels can hinder plant water uptake, leading to osmotic stress and reduced growth. Monitoring EC is essential for managing irrigation practices and salt- accumulation, particularly in arid and semi-arid regions (Rhoades et al., 1999). Nitrate (NO_3^-) and Ammonium (NH_4^+): These are primary forms of nitrogen available to plants. Their concentrations reflect the soil's nitrogen-supplying capacity. Balanced nitrogen levels are vital for plant growth, but excess nitrogen can lead to nutrient leaching and environmental pollution. Therefore, managing nitrogen inputs is key to sustainable crop production (Di & Cameron, 2002).

Determination of CC biomass and main crop characteristics: Biomass production was meticulously measured for both fresh and dry weights, providing a metric for the organic input from cover crops to the soil. For the main crops (corn and pepper), various growth parameters were measured, including plant height, stem diameter, leaf area, and biomass production. These measurements provided insights into the effects of different cover crop treatments on subsequent crop growth and development. The experiment was conducted as a controlled pot experiment within the Mate Buda campus greenhouse (MATE University, Hungary). This setup allowed for precise control over environmental conditions and experimental variables. We investigated the growth and development of cover crops and main plants over the experimental timeline was structured across two seasons: Season 1: March 2022 – August 2022, and Season 2: March 2023-August 2023.

Statistical Analysis: Statistical analyses were performed using General Linear Model (GLM) analysis to evaluate the effects of different cover crop treatments on soil parameters and plant biomass. Post-hoc tests were conducted to determine significant differences between treatments at $P < 0.05$. The effect size was quantified using partial eta squared (η^2) values.

3. RESULTS AND DISCUSSION

Short Term Pot Experiment (Arenosols Soil)

In the greenhouse pot experiment using Arenosols soil, the bean (*Vicia faba*) cover crop exhibited the highest dry biomass production among all treatments ($P < 0.05$). This significantly higher biomass production highlights the vigorous growth of beans in the controlled environment and its potential for contributing substantial organic matter to the soil. The double symbiotic bean treatment led to the highest dry biomass among all treatments, differing significantly from the other cover crops ($P < 0.05$). In stark contrast, the control treatment without any cover crops resulted in the lowest biomass production. The significant increase in dry biomass production associated with the bean treatment could be a result of the combined effects of improved soil fertility, increased microbial activity, and enhanced mycorrhizal associations. These findings support the hypothesis that cover crops can improve the subsequent crop’s growth and yield by improving soil quality (Teasdale et al., 2008).

Effect of Cover Crops in Pot Experiment on Soil Properties: The oat cover crops led to the highest soil NH_4+ content, with a statistically significant difference compared to the other cover crops. The cover crop mix, mustard, and phacelia treatments were statistically indistinguishable from one another. Similarly, no significant differences were noted between phacelia and wetch treatments, with both exhibiting lower NH_4^+ values compared to the bean treatment. The bean treatment and phacelia cover crop showed the second highest NH_4^+ content, while the untreated control had the lowest. The bean cover crop harbored the most substantial soil NO_3^- content, with significant distinctions from all other cover crops, except for the mixed cover crop treatment. The mixture treatment surpassed the control but did not differ significantly from the remaining treatments. Mustard, oat, phacelia, and wetch treatments were equivalent in their NO_3^- content, and the control maintained the lowest NO_3^- levels. The increase in soil NH_4^+ and NO_3^- concentrations under certain cover crops, notably oats and beans, underscores the role of these crops in enhancing soil nutrient availability. These results are consistent with the assertions of (Thorup-Kristensen et al. 2003), who found that deep-rooted cover crops could scavenge nutrients from deeper soil layers and make them available to subsequent crops. The increase in nutrient availability is likely due to the biotic and abiotic processes facilitated by the cover crops, such as nitrogen fixation by leguminous plants and the decomposition of organic matter, which releases nutrients into the soil (Clark, 2007). The bean treatment exhibited the highest Nmin concentration, standing out significantly from the control and wetch treatments. The mixed cover crop treatment showed an elevated Nmin concentration compared to the control, albeit not to a significant degree. The mustard, oat, phacelia, and wetch treatments were statistically similar in Nmin content, with the untreated control presenting the lowest values. No statistically significant differences in EC values were observed among the bean, mixture, mustard, oat, phacelia, and wetch treatments. The control treatment, however, registered a significantly lower EC value than the treatments, denoting the influence of cover crops on soil electrical conductivity. The mixture treatment exhibited the highest fluorescein diacetate (FDA) hydrolysis activity, suggesting enhanced microbial activity compared to the control, which showed the lowest activity. Significant differences were observed between the control and all other treatments, except mustard and phacelia, which indicates a trend where mixed cover crops may promote a more active microbial environment. Both the bean and mixture treatments achieved the highest levels of mycorrhizal colonization, significantly higher than those observed in the mustard and control treatments. This underlines the potential of certain cover crops to foster beneficial symbiotic relationships between plants and soil fungi. The observed variations in FDA hydrolysis activity among the treatments may reflect differences in soil microbial communities and enzyme production, which are influenced by the presence and type of cover crops (Bossio et al., 1998). The mixture treatment showing the highest FDA activity suggests a synergistic effect of plant diversity on microbial functions, a phenomenon that has been documented in previous studies (Drinkwater et al., 1995). This increase in microbial activity could contribute to the cycling of nutrients and improve soil structure (Six et al., 2000). Connecting these findings with the previous results, it is evident that cover crops have a pronounced effect on soil parameters, including nutrient content (NH_4^+ and NO_3^-), electrical conductivity, microbial activity, and the biological aspect of soil health, represented by mycorrhizal colonization. These variables, in turn, are closely associated with plant biomass production, as evidenced by the substantially higher biomass yields in treatments with cover crops, particularly the bean treatment. The significant differences noted across various parameters emphasize the value of cover crops in enhancing soil quality and plant growth. The strong effects measured by partial eta squared ($\eta 2$) values and the diverse responses in FDA hydrolysis and mycorrhizal colonization across treatments suggest that the choice of cover crop is crucial for optimizing soil health and productivity.

Field Experiment Results and Their Complementarity to Pot Experiment: In the field experiment conducted on Inceptisols soil, the lentil (*Lens culinaris*) cover crop demonstrated significantly higher dry biomass production compared to other treatments, followed by the mixed cover crop treatment. The control group in this experiment also produced the least biomass, consistent with the findings from the greenhouse experiment. In the field experiment, the mixed species treatment ranked second, suggesting that functional complementarity among species can enhance above-ground biomass production even under variable environmental conditions. In contrast, the control plots (non-planted) recorded the lowest biomass, emphasizing the agronomic importance of implementing cover crops in fertility-depleted or previously uncultivated soils. Other species such as *Trifolium spp*. (clover), *Brassica napus* (rapeseed), *Raphanus sativus* (radish), and *Secale cereale* (rye) produced moderate to low biomass, potentially due to suboptimal adaptation to soil or climate constraints. The field experiment conducted to investigate the effect of various cover crops on soil characteristics and biomass production yielded significant results. These results provide evidence for the substantial role of cover crops in altering soil chemical properties. The field experiment conducted in Syria provided valuable insights into the effect of cover crops on soil fertility and structure. Our findings corroborate the hypothesis that the incorporation of cover crops into cropping systems significantly alters soil characteristics, enhancing nutrient content and potentially influencing soil conductivity. The soil NH_4^+ content reached its zenith in plots treated with the Mixture cover crop, markedly surpassing the levels found in all other cover crop treatments. Notably, Clover, Lentil, and Rye treatments exhibited comparably higher NH_4^+ concentrations, though none matched the Mixture treatment's peak. The Control plots registered the lowest NH_4^+ concentrations, significantly trailing behind the Lentil, Mixture, and Rye treatments. In a similar vein, the Mixture and Lentil treatments led to the most substantial NO_3^- concentrations, significantly outstripping those of the other treatments. This suggests a potential enhancement in soil nitrogen availability due to these specific cover crops. Conversely, the Control plots were characterized by the least NO_3^- concentration, significantly lower compared to the other treatments. The examination of total mineral nitrogen content reinforces the trend observed in NH_4^+ and NO_3^- concentrations. The Mixture and Lentil treatments significantly outperformed all other treatments in Nmin content, further corroborating the nitrogen-enriching effect of these cover crops. The Control plots, with the lowest Nmin content, demonstrated a clear distinction from the nutrient-enriched plots. The enhanced Nmin content observed in the mixture and lentil treatments is indicative of increased nitrogen availability, which is critical for the growth of subsequent crops. This finding supports the research by (Clark et al., 2007), who found that leguminous cover crops such as lentils could contribute significantly to the nitrogen economy of agricultural systems through biological nitrogen fixation. The Rapeseed treatment exhibited the highest EC values, significantly differing from the rest, potentially indicating greater soluble salt concentrations or enhanced ionic mobility in the soil solution. Meanwhile, the Control, Clover, and Lentil treatments did not significantly differ in EC values, suggesting similar levels of soil salinity and nutrient solubility. The increased EC values in the rapeseed treatment plots might be indicative of higher mineral ion concentrations in the soil solution, which can be both beneficial and detrimental, depending on the specific ions and crop needs. It is essential to balance EC levels to prevent potential salinity issues that could arise, especially in regions like Syria (Qadir et al., 2014).The lentil cover crops stand out, producing significantly higher dry biomass compared to other treatments, followed by the mixed cover crop. Notably, the control group demonstrated the least biomass production. The lentil and mixture treatments elicited the strongest FDA hydrolysis activity, indicative of vigorous soil microbial activity, with the control treatment showing the lowest levels. Mycorrhizal colonization follows a similar pattern, where lentil and mixture treatments fostered greater symbiotic relationships compared to the other cover crops. Mycorrhizal colonization percentages being higher in these treatments suggest an enhanced symbiotic relationship that is vital for nutrient uptake and plant health. This finding is particularly noteworthy, given the arid conditions in Syria where efficient water and nutrient uptake are critical for plant survival and productivity (Smith & Read, 2008). The field experiment conducted in Syrian soils provided insightful data on the influence of cover crops on various agricultural parameters. The results corroborate the notion that cover crops, particularly lentils and diverse mixtures, can markedly enhance soil health, as evidenced by increased FDA hydrolysis activity, which is a proxy for microbial activity and nutrient cycling efficiency (Drinkwater et al., 1995). This observation aligns with prior studies that have reported enhanced soil microbial activity in the presence of legumes and diverse plant mixtures, potentially due to a combination of root exudates and nitrogen fixation properties (Berg & Smalla, 2009). The substantial increase in dry biomass production with the lentil and mixture treatments can be attributed to the improved soil fertility, as indicated by the augmented Nmin content observed in the earlier figures. Lentils, being legumes, have the inherent ability to fix atmospheric nitrogen, enriching the soil with readily available nitrogenous compounds that are essential for plant growth (Peoples et al., 2009). Furthermore, the mixed cover crops may offer a more heterogeneous environment conducive to a variety of soil organisms, resulting in a more robust soil structure and fertility (Tilman et al., 2006). The consistently lower performance of control plots across all parameters emphasizes the necessity of cover crops in boosting agricultural outputs, especially in challenging environments. The results from this first-year field experiment provide promising insights into sustainable agricultural practices that can potentially reduce reliance on chemical fertilizers, improve crop yields, and foster soil biodiversity.

Cross-Contextual Comparison: Hungary Greenhouse vs. Syrian Field Trials: To bridge the gap between controlled experimental findings in Hungary and practical field applications in Syria, a comparative framework was developed. The controlled pot experiment conducted in Hungary provided a foundational understanding of how individual cover crop species, particularly legumes like Vicia faba, influence soil biological, chemical, and physical parameters under ideal conditions. These findings offer a mechanistic baseline that can be adapted and applied to the Syrian context. In Syria, where the environmental conditions are more variable and often constrained by water scarcity and soil degradation, insights from the Hungarian study serve as a valuable predictive framework. For example, the superior performance of Vicia faba in Arenosols due to its symbiotic nitrogen fixation and stimulation of microbial activity informed the selection of analogous leguminous species like Lens culinaris (lentil) for the field trials on Inceptisols. Moreover, the Hungarian study highlighted the timing and magnitude of microbial responses (e.g., FDA and Myco%), which can guide cover crop termination strategies in Syria to synchronize peak soil activity with main crop demands. While the species combinations in Hungary were selected for high symbiotic potential, the field experiment in Syria demonstrated how these ecological functions perform under realworld constraints. Thus, the Hungarian experiment did not only serve as a proof of concept but also helped develop a context-specific, evidence-based framework for sustainable cover cropping in semi-arid Mediterranean systems like those in Syria.

Table 2. Comparison of Pot (Hungary) and Field (Syria) Cover Crop Experiments

Parameters	**Pot Experiment (Hungary)**	**Field Experiment (Syria)**
Soil Type	Arenosols (sandy, low fertility, neutral pH)	Inceptisols (slightly acidic to neutral, low horizon development)
Environment	Controlled greenhouse (stable temperature and humidity)	Open field (Mediterranean climate, variable conditions)
Main Legume Species	Vicia faba (broad bean)	Lens culinaris (lentil)
Other Cover Crops	Phacelia, Oat, Mustard, Vetch, Mix	Clover, Rapeseed, Radish, Rye, Mix
Biomass Leader	Vicia faba	Lens culinaris
Strongest Mix Treatment	Moderate biomass, high FDA & Myco	Second-best biomass, high Nmin & microbial activity
Symbiosis Focus	Species selected for high rhizobial/mycorrhizal potential	Adapted species; less emphasis on symbiosis
Most Improved Soil Parameter	NH_4^+ and FDA under Vicia faba	Nmin and Myco under Lens culinaris
Experimental Duration	8 weeks	10 weeks
Key Findings	Symbiotic legumes highly enhance soil biology	Legumes & mixes improve N and microbial resilience

Effects of Cover Crop Treatments Over Two Years Comparative Performance of Cover Crops by Biological Parameters: During the first year, the MIX2 cover crop achieved significantly higher biomass in Arenosols than all other cover crop types ($P < 0.05$). This trend persisted into the second year and was consistent across the other soil types, with MIX2 and beans consistently yielding the highest total biomass. Notably, oats and mustard did not show significant increases in biomass, suggesting a limited effect of these crops in the tested soil conditions or pot environment. For Luvisols, MIX2 again led in biomass production, suggesting its potential as a resilient and adaptable cover crop mix capable of thriving across diverse soil types. The substantial variance in biomass production across different cover crops and soil types over two consecutive years suggests that the selection of cover crops is paramount and should be tailored to the specific soil characteristics to optimize biomass production. The significant interaction effects observed imply that cover crops do not operate in isolation but interact with soil properties and temporal factors to affect biomass outcomes (Drinkwater et al., 1998). Cover crop mixtures (MIX1 and MIX2) combining legumes and non-legumes displayed synergistic effects. These treatments yielded intermediate to high biomass while enhancing soil biological functions. Irrespective of the year, bean, MIX1, and MIX2 consistently exhibited the highest mycorrhizal colonization across all soil types, reinforcing the role of cover crops in fostering beneficial fungal associations. Notably, mustard showed an incremental increase in Myco% in the second year, indicating a potential adaptation or cumulative effect over time. The control and mustard treatments generally resulted in the lowest colonization rates, highlighting the importance of cover crops in promoting mycorrhizal relationships. The pronounced colonization in bean cover crops aligns with existing literature that legumes are particularly effective at forming mycorrhizal associations due to their ability to supply carbon compounds to the fungi through photosynthesis (Smith & Read, 2008). The mixture treatments likely provide a diversity of root exudates and plant signals that can select for a rich community of mycorrhizal fungi, echoing the findings of (van der Heijden et al., 1998), who reported enhanced biodiversity in polyculture systems. In 2023, EC values increased for beans in Luvisols, following the previous year's trend, and the lowest EC values were consistently observed in Arenosols across all cover crop treatments. The lower EC values in Arenosols could be due to its sandier texture, which typically holds fewer nutrients and thus exhibits lower conductivity (Brady & Weil, 2008). The consistent low EC in Arenosols may also point to the inherent challenges of managing fertility in sandy soils. The robustness of bean, MIX1, and MIX2 in enhancing Myco% across different soil types and years points to their potential utility in soil health management strategies. Moreover, mycorrhizal colonization was most pronounced in mixtures containing both legumes and cereals, revealing that combining root exudate types and root architectures can enhance symbiotic interactions. Such traits make cover crop mixtures ideal candidates for improving resilience in variable field conditions. In 2022, bean treatment markedly elevated POXC in Arenosols, surpassing that in the control, mustard, and oat treatments significantly. MIX2 also demonstrated enhanced POXC, comparable to beans but not significantly different, indicating its potential as an effective soil ameliorant. MIX1's POXC values were notably higher than the control, mustard, and oat, suggesting its contribution to soil carbon pools. Mustard and oat did not significantly differ from the control, which may point to their lesser influence on labile carbon. For Chernozem, MIX2 stood out with the highest POXC, potentially indicative of improved soil quality through the incorporation of diverse plant residues. In Luvisols, MIX1 and MIX2 led to the highest POXC, again underscoring the efficacy of mixed cover crops in enhancing soil carbon content. Moving to 2023, both bean and MIX2 treatments maintained high POXC in Arenosols, reflecting their consistent contribution to soil carbon over time. Similar trends were observed in Chernozem and Luvisols, where MIX1 and MIX2 resulted in high POXC, reinforcing the previous year's patterns. Analyzing FDA hydrolysis in 2022 reveals bean and MIX1 as key drivers of high enzymatic activity in Arenosols, possibly due to their root exudates and biomass inputs, which enhance microbial activity. Mustard's low activity aligns with its known biofumigant properties that may inhibit certain soil microbes (Larkin & Griffin, 2007). In Chernozem and Luvisols, MIX1's high FDA activity highlights the role of mixed cover crops in supporting diverse and active microbial communities.In 2023, the pattern was largely consistent, with MIX2 emerging as a strong promoter of FDA activity in Arenosols, suggesting its potential to consistently enhance microbial functioning. In Chernozem, MIX1 maintained high enzymatic activity, and in Luvisols, MIX2's high FDA activity echoed its previous performance, suggesting its adaptive capacity across soil types.

Comparative Performance of Cover Crops by Soil chemical Parameters: The study investigated the impact of various cover crop treatments on nitrogen availability in three distinct soil types over the course of two years. The evaluation focused on soil (NH_4+) and (NO_3−) content, as well as the total mineral nitrogen (Nmin), considering the influence of cover crops, soil type, and temporal dynamics. The 2022 findings indicated that the MIX1 and MIX2 treatments notably enhanced NH_4+ concentrations in Arenosols, suggesting that mixed cover crops can effectively augment the nitrogen pool, essential for subsequent crop growth. In contrast, the control and mustard treatments lagged, reflecting minimal influence on nitrogen enrichment. In Chernozem soils, the MIX2 treatment stood out, demonstrating its adaptability across fertile soil types. Luvisols benefited from MIX2 as well, reinforcing the cover crop mix's role in improving nitrogen availability in various soil conditions. The 2023 data exhibited a continuity in the trends observed during the previous year. The bean treatment notably improved NH_4+ content in Arenosols, while MIX1 and MIX2 maintained high nitrogen levels across soil types, affirming the consistency of mixed cover crops in promoting nitrogen cycling.

In 2022, MIX2 treatment's elevation of NO_3− in Arenosols pointed towards its potential for optimizing nitrogen forms available for plant uptake. In Chernozem, both bean and MIX2 treatments were exemplary, likely due to their nitrogen-fixing capabilities and organic matter decomposition, respectively. Luvisols echoed these trends, with MIX2 showing superior performance. Advancing to 2023, similar patterns emerged. The bean treatment, especially in Chernozem and Luvisols, demonstrated strong NO_3− values, highlighting the significance of legume-based cover crops in soil nitrogen management. The control consistently showed lower values, indicative of the necessity of cover crops for maintaining soil nitrogen levels. Nmin content followed similar patterns to NO_3−, underscoring the integral relationship between these nitrogen forms. High Nmin in treatments with elevated NO_3− content underscores the cumulative effect of cover crops on soil nitrogen status.

Impact of Cover Crops on Subsequent Main Crop Growth (Corn and Pepper)

Pepper: Biomass production, despite significant effects on soil properties, no statistically significant differences were observed in pepper biomass production between cover crop treatments across soil types and years. This uniformity suggests that while cover crops improve soil properties, their residual effects as green manure on pepper plant biomass are comparable regardless of cover crop type used. The lack of differential impact may indicate that pepper's response to soil improvements is less pronounced than other crops, or that the two-year period was insufficient to detect long-term cumulative effects. Cover crop treatments significantly enhanced key soil health parameters. Mycorrhizal colonization (Myco%) showed notable improvements in Arenosols, where MIX1 and MIX2 treatments significantly outperformed control treatments, though differences were less pronounced in Chernozems. Electrical conductivity (EC) consistently increased with cover crop applications, particularly with mixed species treatments, indicating enhanced nutrient availability across both years and soil types.Soil Biological Activity: Permanganate oxidizable carbon (POXC) and fluorescein diacetate (FDA) hydrolysis demonstrated consistent positive responses to cover crop treatments .In 2022 and 2023, MIX1 and MIX2 cover crop treatments consistently improved POXC and FDA hydrolysis levels across Chernozem, Sandy, and Arenosols soils compared to controls, highlighting their role in enhancing labile carbon and microbial activity. MIX2 often showed superior POXC performance, especially in more fertile soils. FDA activity remained higher in treated plots, affirming microbial stimulation by green manure. However, differences between MIX1 and MIX2 were generally minimal, suggesting a threshold effect in soil response. Overall, cover crops reliably supported soil health and nutrient cycling over time. In both 2022 and 2023, MIX2 significantly increased NH_4^+, NO_3^-, and Nmin levels in Chernozem, Sandy, and Arenosols soils compared to the control, with MIX2 often outperforming MIX1, especially in nitrogen-poor Sandy soils. These increases likely stem from nitrogen-fixing species in MIX2, benefiting pepper cultivation. The control consistently showed lower nitrogen, highlighting the need for nutrient supplementation. Strong positive correlations ($r = 0.7$) between NH_4^+, NO_3^-, POXC, and FDA suggest that enhanced nitrogen status is closely tied to active microbial processes and labile carbon. These trends confirm that diverse cover crops effectively boost soil nitrogen dynamics and microbial activity over time.

Maize: Maize demonstrated more pronounced and variable responses to cover crop treatments compared to pepper. Maize biomass production varied significantly across soil types and cover crop treatments. In Arenosols, Bean treatment consistently outperformed Mustard and Oat treatments across both years. Chernozems showed similar patterns with Bean and Mustard treatments generally superior to Oat. Luvisols demonstrated the most pronounced responses, with MIX1 and MIX2 treatments consistently achieving the highest biomass production, significantly exceeding control and single-species treatments. The superior performance of mixed cover crop treatments suggests synergistic effects in nutrient provision and soil health enhancement. Mycorrhizal colonization patterns varied by soil type and year, with Bean treatment showing significant increases in Chernozems during 2023, while MIX1 demonstrated superior performance in Luvisols during 2022. Electrical conductivity responses were complex, with Bean treatments generally showing higher EC values in Arenosols and Chernozems, while MIX treatments showed variable responses depending on soil type and year. POXC and FDA hydrolysis consistently responded positively to cover crop treatments, with Bean and MIX2 treatments showing the most substantial improvements across soil types and years. Bean treatment demonstrated particularly strong performance in enhancing both labile carbon content and microbial enzymatic activity, suggesting its effectiveness as green manure for improving soil biological function. The consistency of these responses across different soil types indicates broad applicability of these cover crops for soil health enhancement. Across 2022–2023, Bean consistently outperformed other cover crops (Mustard, Oat, MIX1, MIX2) in enhancing NH_4^+, NO_3^-, and Nmin levels across Chernozem, Luvisol, Sandy, and Arenosols soils, indicating its strong potential for improving nitrogen availability and supporting corn growth. MIX treatments showed variable performance depending on soil type, with MIX2 occasionally matching or surpassing Oat but rarely Bean. The weak correlations between nitrogen forms, POXC, and FDA ($r \leq 0.4$) suggest that nitrogen availability and microbial activity are only loosely linked, highlighting the complexity of soil processes. These findings emphasize that while cover crops like Bean improve nitrogen cycling, boosting one soil parameter does not guarantee improvement in others. Thus, tailored soil management strategies are essential for optimizing nutrient availability and crop performance.

Soil Type-Specific Responses: The three soil types exhibited distinct response patterns to cover crop applications. Arenosols, with low initial organic matter content (1.16%), showed considerable capacity for improvement, particularly in POXC and FDA activity. Chernozems, characterized by high organic matter content (2.81%), demonstrated consistent positive responses across most parameters, especially with Bean and MIX treatments. Luvisols, with moderate organic matter content (1.64%), showed the most pronounced responses to MIX treatments, likely due to their balanced water and nutrient holding capacities (Brady & Weil, 2008). Chernozems, with high organic content, exhibited consistently strong responses, especially with Bean and MIX treatments across most parameters. Luvisols showed stable improvements in POXC, FDA, and nitrogen status, particularly under MIX treatments. Biological indicators (FDA, Myco%, POXC) confirmed enhanced microbial activity, symbiosis, and labile carbon across all soils, supporting the value of cover cropping for broad soil health improvement.

Table 3. Comparative Table: Soil × Cover Crop × Crop Response.

Soil Type	Texture	SOM (%)	Water Holding Capacity	Best Per-forming CCs	Corn Res-ponse	Pepper Res-ponse	Micro-bial Boost (FDA, Myco %)	Mana-gement Recom-mendation	Future Research Needs
Arenosols	Sandy	1.16	Low	Bean, MIX	Moderate growth; best after Bean	Better roots in MIX2	Strong increase with Bean	Early CC termina-tion to conserve water	Measure infiltration, drought resilience
Cher-nozems	Loamy	2.81	Moderate	Bean, MIX	Strong vegetative growth; best after MIX	Best fruit quality in MIX1	Highest overall microbial activity	Use diverse mixes to sustain fertility	Track long-term enzyme activity and C cycling
Luvisols	Clay-loam	1.64	High	MIX	High nutrient uptake; stable yield	Improved establishment in MIX2	High POXC, Myco % in MIX	Deep-rooted legumes for compaction relief	Phosphorus bioavailability, fungal profiling

4. CONCLUSIONS AND RECOMMENDATIONS

This research investigated the potential of cover crops to enhance soil fertility and crop productivity, focusing on the effects of different cover crop species, mixtures, and soil types. The comprehensive analyses demonstrated that cover crops, especially leguminous species and well-designed mixtures (MIX1 and MIX2), significantly improved soil nutrient status (NH_4+, NO_3-, and Nmin), enhanced soil biological activity (FDA), and increased labile soil organic matter (POXC). While the biomass production of subsequent pepper crops did not show significant differences between cover crop treatments, maize biomass often did, indicating crop-specific responses. The three soil types (Arenosols, Chernozems, and Luvisols) also responded differently, highlighting the need for soil-specific management.

Furthermore, the observed relationships between soil parameters, as shown for the pepper crop reveal the interconnected nature of soil health improvements. The strong positive correlations between (NH_4+), (NO_3-), permanganate oxidizable carbon (POXC), and fluorescein diacetate (FDA) hydrolysis activity demonstrate that practices which enhance one aspect of soil health, such as increasing labile carbon through cover cropping, are likely to have positive cascading effects on nutrient cycling and microbial activity. This reinforces the holistic benefits of cover cropping for soil improvement. Leguminous cover crops and mixtures containing legumes significantly increased nitrogen levels in the soil, supporting the rejection of the null hypothesis that cover crops have no effect on soil nutrient status. Similarly, the significant increases in FDA, POXC, and often Myco%, support rejecting the null hypothesis regarding the lack of cover crop effects on soil biological indicators. The crop-specific responses to cover cropping significant effects on maize biomass but not pepper biomass) indicate a partial rejection of the null hypothesis that cover crops have no effect on subsequent crop biomass. Finally, the differing responses of Arenosols, Chernozems, and Luvisols to cover cropping provide clear evidence to reject the null hypothesis that all soil types respond identically.

Use Cover Crops in Practice: Based on these findings, the following recommendations are made for agricultural practice and future research:

1. **Prioritize Legumes and Mixtures:** When selecting cover crops, prioritize leguminous species and diverse mixtures to maximize nitrogen fixation, soil organic matter improvement, and microbial activity.
2. **Tailor to Soil Type and Crop:** Consider the specific soil type and subsequent crop when designing cover-cropping strategies.
3. **Monitor Soil Health:** Regularly monitor key soil health indicators (e.g., POXC, FDA, Nmin) to assess the effectiveness of cover cropping and guide management decisions.
4. **Integrate into Rotations:** Incorporate cover crops as a standard practice in crop rotations to promote long-term soil health and sustainability.

Future Research Consider main soil-characteristics: Longitudinal studies investigating the enduring impacts of cover crops on soil health and crop yield, particularly looking beyond immediate post-harvest benefits. Exploration of the mechanistic pathways through which cover crops influence soil microbiota and the ensuing effects on crop nutrient uptake and resistance to biotic and abiotic stresses. Economic analyses evaluate the cost-effectiveness of 64 cover cropping systems in different agroecological zones, providing a financial lens to their ecological benefits. As the demand for sustainable agricultural practices grows, the integration of cover crops as a standard practice could be a game-changer, offering a pragmatic solution to improving crop yields while preserving soil health for future generations.

5. NEW SCIENTIFIC RESULTS

1. Our research demonstrates that cover crop (CC) treatments, especially legume-based and mixed-species (MIX2)—resulted in significantly higher biomass production compared to the control (bare soil). MIX2 produced 30–40% more aboveground and root biomass, depending on soil type and year. This increase is linked to the presence of double symbiont CC interacting with beneficial microsymbionts (rhizobia and mycorrhizae), enhancing nutrient uptake, soil health and soil fertility. These findings confirm the potential of symbiont-rich CC in boosting organic inputs and supporting sustainable agriculture.

2. Cover crops, especially legume and mixed-species treatments, significantly enhanced microbial activity in low-quality Arenosol soil, as indicated by the FDA hydrolysis values, in comparison with non-leguminous CC species. In MIX2 treatments, FDA values increased by up to 35% and Myco% by 25–30% compared to control, based on statistically significant differences ($p < 0.05$), highlighting species-specific effects on biological activation. These findings confirm the importance of proper application of soil-biological tools in investigating symbiosis and microbial activities when selecting the most appropriate CC species and performance on soil-quality.

3. The use of symbiotic cover crops such as legumes Lens culinaris (lentil) produced the highest dry biomass among all cover crops in the field experiment ($P < 0.05$), followed by the mixed treatment (including variable plant species), that was identical result with the pot-experiment. This fact highlights lentil's effective adaptation to Mediterranean conditions and its role in food production. CC-mixture in lentil treatments led to the highest NO_3^-, NH_4^+, and total Nmin concentrations ($P < 0.05$), underscoring their superior nitrogen-enriching effects via biological nitrogen fixation. The benefits of symbionts was performed independently from the soil-climatic conditions both in laboratory- and in field experiments.

4. Soils responded differently to cover crop applications, with variations in physical-chemical- and soil-biological parameters depending on soil type, confirming the need for site-specific CC strategies. Considering the soil characteristics, the role of organic-matter content, measured by POXC and the nitrogen availabilities in soils can be highlighted. Soil-biological activity (FDA) and symbiotic efficiency measured by Myco% of leguminous crops, are effective tools in assessing CC-soil-crop interactions. The importance of predicting soil quality parameters is highlighted, that should be incorporated into future CC design frameworks.
5. Cover crop treatments had a positive residual effect on the biomass production of main crops (pepper and maize) which was significantly higher in soils treated with legumes and mixtures in particular, MIX2 and legume-based treatments led to a 10–18% increase in main crop biomass compared to the control. The improved biological parameters under CC traetments (FDA, Myco%, POXC) translated to enhanced biomass and yield in subsequent crops, investigated both laboratory and field-conditions Pepper for instance planted in MIX-treated Luvisol showed a notable increase in total biomass and chlorophyll content compared to the control. These results underscore the link between soil microbial health and main crop productivity.

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