CORE Organic Cofund

"SCOOP: Developing intercropping systems with camelina to increase the yield and quality parameters of local underutilized crops"

Deliverable report

D 2.5. Report on ecosystem services related to SCOOP intercropping systems

Authors: Reyhan Bahtiyarca, Çağlar Sağun, Dilek Kaya, Aynur Dilsiz, Emel Çakır, Cenk Yücel, Işıl Özdemir, Federica Zanetti, Michał Krzyżaniak, Makowski Przemysław, Kordan Bożena, Kosewska Agnieszka, Marina Marcheva, Mariana Andonova

Central Research Institute of Field Crops,

Ankara, Türkiye

March, 2025

CONTENTS

1. INTRODUCTION

The SCOOP project is dedicated to developing innovative and diverse organic intercropping systems with the goal of preserving the integrity of ecosystems and agricultural land, enhancing biodiversity, and ensuring food and feed security. This new intercropping approach will be centered around camelina (*Camelina sativa* L. Crantz), a versatile European oilseed, that is capable of maintaining consistent yields under rainfed conditions across various regions in Europe.

Companion crops for camelina, such as pulses (e.g., pea, lentil, fenugreek, vetch), pseudo-cereals (e.g., buckwheat), or ancient cereals (e.g., spelt), were monitored regarding their above/below ground biodiversities with the scope of the present project during the years of 2021 and 2024.

Project results confirmed that Intercropping systems offer significant ecosystem services due to their inherent symbiotic nitrogen-fixation abilities (regarding legumes) and their contribution of nitrogen to succeeding crops, as well as phosphorus remobilization facilitated by mycorrhiza and soil bacteria. These processes lead to reduced external nitrogen and phosphorus inputs, lower energy consumption, enhanced biodiversity, and overall soil improvement.

Particularly in the difficult circumstances of arid and semi-arid countries, different intercropping combinations have the ability to improve water-use efficiency, decrease the usage of pesticides and fertilizers, and raise yield production. Along with reducing damage from pests, diseases, and weeds, it will also enhance ecological balance, product quantity, and quality. Through biological nitrogen fixation, legumeous plants in intercropping systems dramatically increase soil fertility, highlighting the many advantages of this technique (Dai et al., 2019).

The current intercropping system demonstrated improvements in soil nutrients and microbiological composition, including macro and micronutrients, organic matter, and mycorrhiza inoculations. Mycorrhizal fungi enhance the uptake of water and nutrients by plants from the soil, increase plant resilience to various environmental stressors, and stimulate microbial activity. Furthermore, these fungi play a vital role in the process of soil aggregation.

Intercropping has been shown to be an effective approach for reducing nematode damage and disease in agricultural fields. On average, it leads to a 40% decrease in nematode-related effects and a 55% reduction in disease-related impacts on the primary crop's performance (Chadfield et al. 2022).

Therefore, camelina intercropping provides multiple benefits, including: i) early soil coverage, which enhances the efficient use of natural resources, prevents nitrogen leaching, and reduces soil erosion; ii) reduced inputs for pest and disease control due to the higher competitive advantage of diversified cropping systems over weeds and diseases; iii) improved seed quality traits of the produced crops; iv) new market opportunities; v) prevention of biodiversity loss and restoration of degraded ecosystems in line with the "Biodiversity Strategy 2030.

Present report analysis the three-year project findings of SCOOP partners (CRIFIC, UWM, UNIBO, and AUP), evaluating above- and below-ground biodiversity in relation to sole-cropping and intercropping of underutilized crops. These crops were cultivated under organic farming practices, sown during autumn and spring alongside camelina.

2. CRIFIC's FINDINGS

2.1. Soil Ecosystem Services

2.1.1. Soil Physical Parameters and Climate of The Experimental Area

The average annual precipitation in Haymana during the experimentals were 258,2 mm, the average temperature 13,2 ºC, and the humidity was 55,4%. The experiments were established as autumn and spring sowings in the successive three growing seasons of 2021-2024.

Soil samples were taken from the experimental area, before sowing and after harvest to determine physicochemical parameters (including EC, pH, available phosphorus, available potassium, organic material, lime contents and *Bouyoucos % sand*, clay, silt, and texture analyses). The experimental soil was classified as loam-clay loam. The lime ratio was high (26,99-27,30%), the organic matter was low (0,94-1,43%), the available phosphorus was medium (14,3-30,1 kg/ha) and the pH level exhibited slight fluctuations over the years, ranging between 7.88 and 8.07, maintaining a generally mildly alkaline soil condition.

2.2. Seed Materials and Experimental Lay out

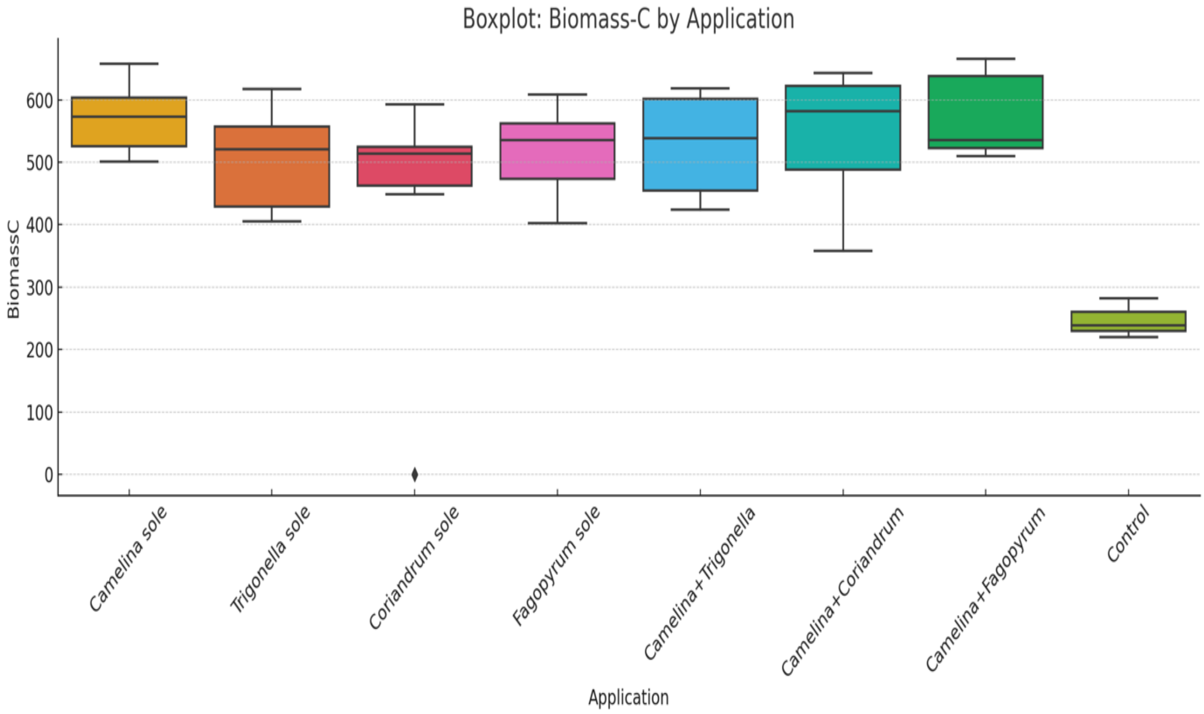
Camelina (*Camelina sativa*) was the main crop, and the companions were fenugreek (*Trigonella foenum graecum*), coriander (*Coriandrum sativum*), and in addition those buckwheat (*Fagopyrum esculentum*) was included in spring sowings. Local varieties were used in the experimental such as; CV Çiftçi (fenugreek), CV Arslanbey (Camelina), CV Sancar Bey (Coriander), and CV Güneş (Buckwheat). The experimental design was laid out compatible with a randomized complete block design technique, with 4 replicates. The quantity of seed rates was 50:50 in intercropping parcels. Plot sizes were 5x5=25 m^2, by 17 cm.

2.3. Soil Microbial Biomass Carbon

Soil microbial biomass carbon (Biomass-C) is a critical indicator of soil health and ecosystem stability, reflecting the turnover of organic matter and microbial activity within the soil (Jenkinson & Ladd, 1981). The diversity and composition of plant species influence soil microbial communities through mechanisms such as root exudates, organic matter inputs, and symbiotic interactions, thereby affecting Biomass-C levels (Zhang et al., 2024). Microbial diversity was assessed using indices such as Shannon, ACE, and Chao1. Principal Coordinate Analysis (PCoA) was conducted to evaluate dissimilarities in phylogenetic distances, based on UniFrac analysis. Mycorrhizal inoculation was examined both visually and through soil DNA extraction techniques.

Among the five tested crop patterns, '**camelina sole,' 'camelina + coriander,' and 'camelina + buckwheat**' proved to be the most effective in promoting soil carbon sequestration and microbial activity, as depicted in Figure 1. These combinations consistently displayed higher and more stable Biomass-C levels, underscoring their significant potential to enhance soil carbon storage and boost microbial activity. The findings for *Camelina sativa*-based intercropping systems highlight their ability to positively impact microbial communities and carbon storage, potentially through mechanisms like root exudates or symbiotic relationships.

Figure 1. The Biomass-C fluctuations of the combinations over the three years (2021 to 2024)



While different intercropping combinations showed year-to-year variability in Biomass-C levels, influencing by environmental conditions, soil nutrient availability, and annual climate changes. Among these, *Camelina sativa* + *Coriandrum sativum* and *Camelina sativa* + *Fagopyrum esculentum* displayed the greatest median values of soil microbial biomass carbon (Biomass-C). These combinations exhibited broader distributions in the boxplot analysis, reflecting not only enhanced biological effectiveness but also adaptability to fluctuating conditions. On the other hand, monoculture systems like camelina sole and fenugreek sole showed lower yet relatively stable Biomass-C values, marked by tighter ranges and reduced medians, which indicate a moderate but consistent impact on soil microbial dynamics. Meanwhile, the control group, (empty plot), consistently demonstrated the lowest Biomass-C values with minimal variability, emphasizing its limited ability to promote microbial activity and carbon sequestration.

These findings are consistent with earlier research on the impact of plant diversity and symbiotic relationships on soil carbon cycling. Studies by Dijkstra et al. (2006) and Cong et al. (2015) highlighted that intercropping legumes by oilseeds, effectively boost microbial activity and promote soil carbon storage through rhizosphere interactions. This combination shows great potential for contributing to the improvement of long-term soil health. Conversely, the variability observed in ***Fagopyrum esculentum*** combinations that could be attributed to this species' sensitivity to environmental changes or its interactions with soil microbial communities.

This analysis underscores the critical role of **selecting appropriate plant combinations to optimize soil health and sustainability in agricultural systems**. Future investigations should delve into the biochemical and microbial processes underlying these patterns, considering additional soil parameters such as enzymatic activity, nitrogen cycling, and organic matter decomposition rates.

2.4. Soil Biodiversity Analysis

2.4.1. Soil DNA extraction and Mycorrhizal denaturing gradient gel electrophoresis (DGGE)

To evaluate the impact of intercropping systems on arbuscular mycorrhizal fungi (AMF) communities, DNA was extracted from soil samples, and AMF communities were amplified using polymerase chain reaction (PCR) targeting 18S rRNA gene regions (Redecker et al., 2003). The amplified DNA samples were then analyzed through **Denaturing Gradient Gel Electrophoresis (DGGE)** to assess AMF community structure. Mycorrhizal inoculation was evaluated both through visual inspection and soil DNA extraction.

Arbuscular Mycorrhizal Fungi (AMF) are essential microorganisms that form symbiotic relationships with plant roots, enhancing nutrient uptake and playing a critical role in ecosystem functions. They facilitate the availability of essential nutrients, particularly phosphorus, which is often difficult for plants to absorb (Smith & Read, 2008). The impact of different agricultural practices on AMF community composition is a key factor in maintaining agricultural biodiversity and soil health (Verbruggen et al., 2012). Monoculture and polyculture systems have varying effects on AMF diversity, with monoculture systems often reducing microbial diversity, whereas polyculture cropping systems tend to support and sustain AMF diversity (Lehmann & Rillig, 2015). The effectiveness of AMF in promoting agricultural sustainability depends on the plant species and agricultural practices employed (Zubek et al., 2010).

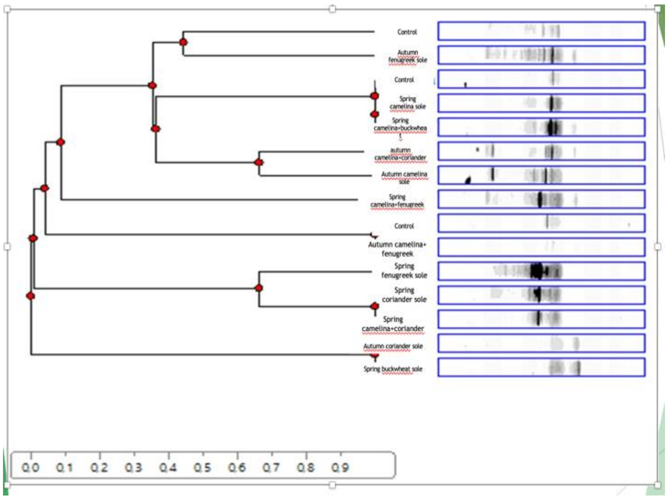
2.4.2. UPGMA (Unweighted Pair Group Method With Arithmetic) Analysis

To determine an accurate way of arranging a group of taxa in a phylogenetic tree, a UPGMA, an agglomerative hierrarchical clustering method was also implemented. The dendrogram shows the phylogenetic similarities and differences between the arbuscular mycorrhizal fungi (AMF) community structures under different agricultural practices (Figure2-5). The dendrogram reveals significant differences between the autumn and spring crop rotations. Soil samples were taken from the rhizosphere. DNA was extracted from the soil samples, and AMF communities were amplified using polymerase chain reaction (PCR) with 18S rRNA gene regions (Redecker et al., 2003). The amplified DNA samples were then separated using Denaturing Gradient Gel Electrophoresis (DGGE), and the band profiles obtained were used to analyze the structure of the AMF communities. AMF community structure; 2022,2023, 2024.As demonstrated in the analysis, the applications are grouped into two main clusters based on AMF band diversity.

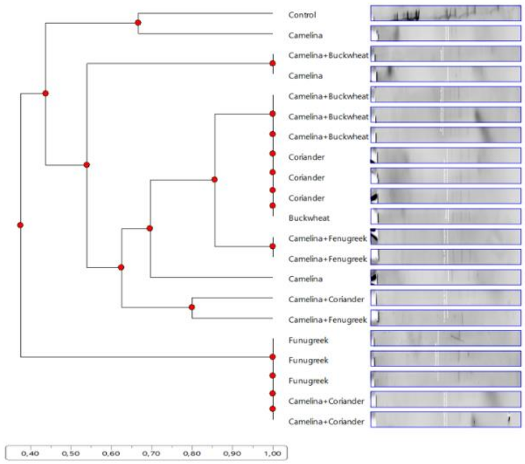
The analysis revealed two main clusters based on AMF band diversity. Camelina sativa and Camelina + Coriandrum sativum formed a distinct cluster separate from other treatments. The remaining treatments were further divided into two subclusters: one included the control and Camelina sativa treatments, while the other was split into two additional clusters. In one part, Camelina + Coriandrum and Camelina + Camelina were grouped together. In the other part, Camelina sole, Camelina + Fagopyrum, Coriandrum, and sole Fagopyrum formed another subcluster. The band intensity analysis indicated that AMF species diversity varied across treatments.

Figure 2, 3, 4, 5. Figure 1 Shows the Effects of Different Intercropping Methods on Arbuscular Mycorrhizal Fungi Community Structure of 2022 autumn sowings, 2 shows 2023's spring sowings, 3 2023's autumn sowings and 4 2024's spring sowings of SCOOP.

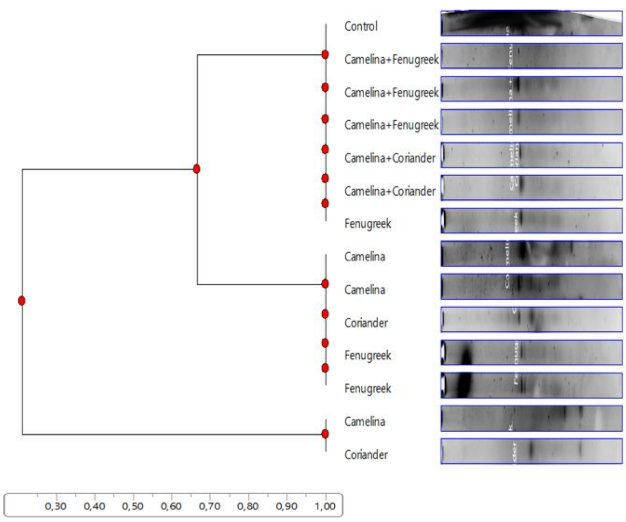
2



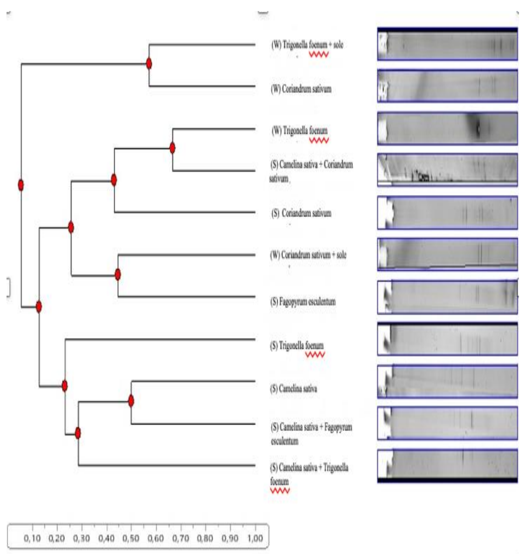
3



4



5



DGGE results showed differences in AMF community structure between autumn and spring sowings. **AMF diversity in spring sowing was found to be superior** compared to autumn sowings. It's recorded that this may caused due to the glucosinolates (GSLs) and dimethyl disulfide content of *Camelina sativa* believed to hydrolyze into isothiocyanates during decomposition (Walsh et al., 2014). These compounds may induce a biofumigation effect, reducing pathogen inoculum in the soil. However, this biofumigation effect may also have a positive impact on beneficial soil microorganisms, including AMF (Walsh et al., 2014).

2.4.3. DGGE (Denaturing Gradient Gel Electrophoresis) Band Profiles

Spring sowing crops revealed a steady rise in microbial diversity over the time, particularly in *Camelina sativa, Trigonella foenum-graecum*, and their combinations, indicating progressive enrichment of microbial communities (Figure 6). On the other hand, autumn sowing crops exhibited greater variability, with microbial activity negatively impacted in 2024 due to winter damage observed in *Coriandrum sativum* (Figure 7). However, certain combinations, such as *Camelina* + Fenugreek, consistently displayed high band counts across both seasons, highlighting the role of plant diversity in fostering microbial diversity. The more stable and upward trends in microbial activity during spring suggest that seasonal environmental factors, such as temperature and soil moisture, are more conducive to microbial development during this period. These findings emphasize the importance of integrating sowing time and plant combinations to enhance soil microbial diversity and support sustainable agricultural practices.

Figure 6. The Average DGGE Band Trends of SCOOP Crops in Spring Sowings of during the years of 2021 and 2024

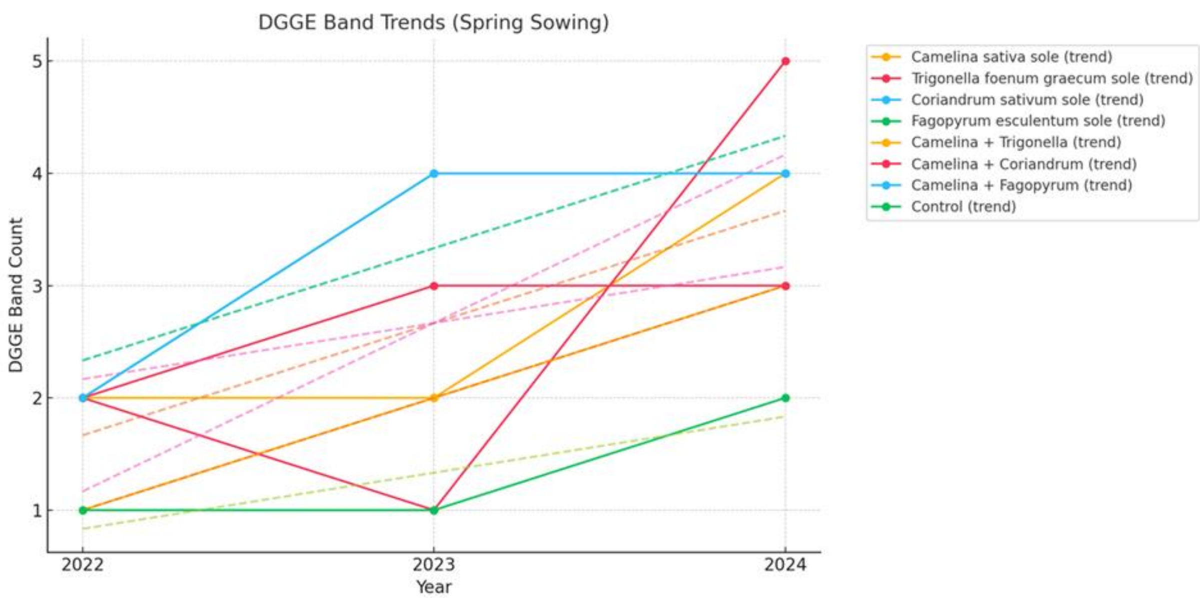
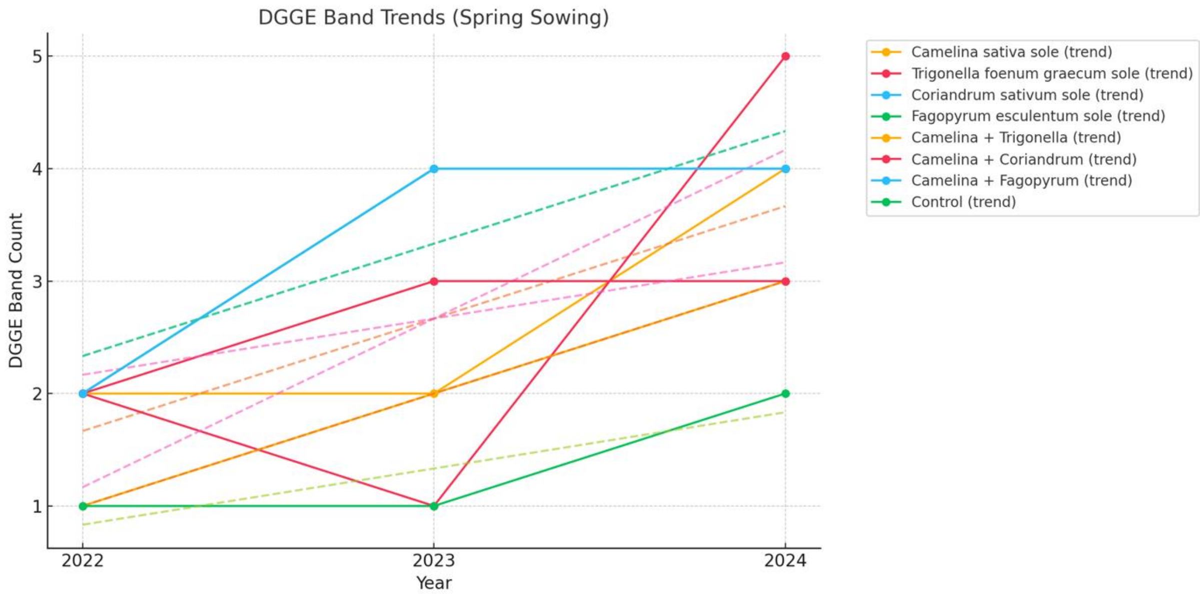


Figure 7. The Average DGG Band Trends of SCOOP Crops in Autumn Sowings of during the years of 2021 and 2024

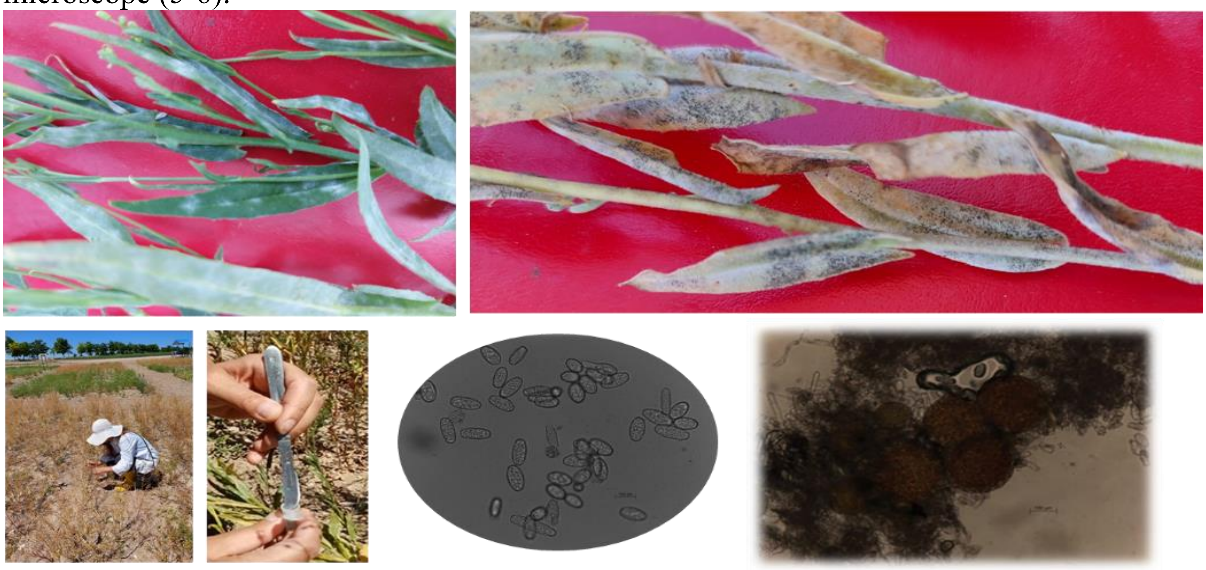


2.5. Plant Disease-mediated Ecosystem Services

Disease identification began in the laboratory prior to seed sowing. Each seed undergoes a thorough physical inspection, where its cleanliness -free from inert matter, weeds, and other crop seeds- is verified, and its germination capacity is assessed. The seeds that underwent these processes were then utilized for planting.

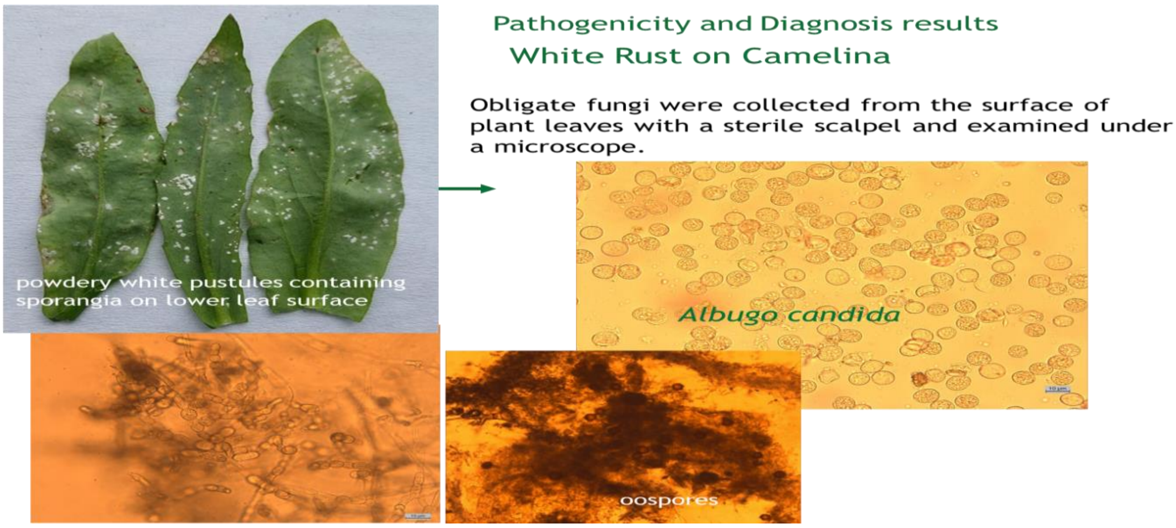
The initial year of the study (2021–2022), powdery mildew (*Erysiphe cruciferarum*) emerged as the most critical challenge during the growing period for camelina plants, resulting in a severe epidemic (Image 1-6). The disease was detected in 100% of all plots. It affected camelina plants in both sole and intercropped plots, with mixed sowing showing no reduction in the disease. Furthermore, the disease was also observed in some coriander plants in mixed sowings of camelina and coriander.

Image 1.-6. The images of powdery (*Erysiphe cruciferarum*) at the field (1-4) and under the microscope (5-6).



In intercropping of coriander and camelina, parcels this wilt wasn't observed. However, in June of the same year, camelina plants began showing symptoms of yellow spots, accompanied by a white fungal layer on the underside of the affected areas. *Albugo candida*, an obligate fungus responsible for white rust (Image 7-10), was identified through isolations conducted on these spots. This pathogen, which affected camelina plants, was not found in coriander, buckwheat, or fenugreek in mixed sowings. Research indicates that camelina is particularly susceptible to white rust (Séguin-Swartz et al., 2009).

Image 7.-10. White rust image (7) and microscope images (8-10) of *Albugo candida*



The climate conditions between April and June 2023 were highly unusual, characterized by unusually cold weather and nearly continuous rainfall. The earliest symptoms of the disease appeared in coriander plants as brown spots on the lower leaves in April. By May, the infection had progressed, and *Alternaria alternata* was isolated from the affected spots.

Image 11-14. Alternaria black spot image (11) and microscop images (12-14)

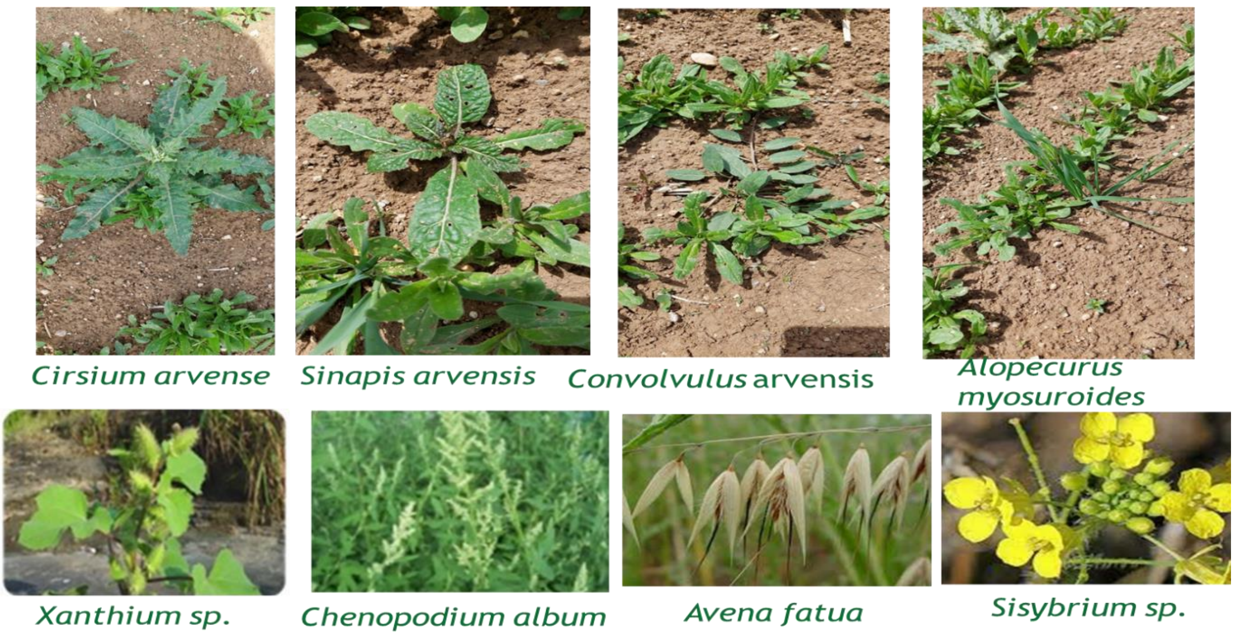
Isolations from the dark brown Alternaria spots typical on camelina plants led to the identification of *Alternaria brassicae* in early July. This pathogen, *Alternaria brassicae* (Berk.) Sacc., causes Alternaria black spot (Figure 11-14), a destructive disease affecting many crucifer crops globally (Verma and Saharan, 1994). The symptoms vary with environmental conditions and usually manifest as stem and leaf spots ranging in color from gray to black. Meanwhile, in sole coriander plots, soilborne pathogens such as *Fusarium equiseti*, *Fusarium acuminatum*, *Epicoccum nigrum*, and *Epicoccum layuense* were also identified molecularly, to be 40% less prevalent in plots where coriander was intercropped with camelina.

By late May to early June of 2024 (01.06.2024), powdery mildew symptoms appeared as white spots on the lower leaves of camelina plants across all plots. The disease was particularly prominent in plots where camelina was intercropped with coriander. Microscopic analysis of the fungal layer collected with a sterile scalpel confirmed the presence of *Erysiphe cruciferarum*. In contrast, no disease symptoms were observed in fenugreek or buckwheat. Additionally, cold damage, especially in camelina, particularly in autumn sowings plots, led to emergence problems during this period.

Regarding the larger scale production areas, the presence of the obligate fungus *Albugo candida,* responsible for white rust was observed in July 2024. Symptoms began as yellow spots on the leaves of camelina plants, however due to subsequent of the dry weather conditions the disease remained confined to the affected plots and did not escalate into an outbreak.

Among all the crops studied, camelina was found to be the most susceptible to diseases which followed by coriander. On the other hand, fenugreek and buckwheat remained disease-free throughout the studies. Intercropping had no impact on the emergence of pathogens like *Albugo candida* and *Erysiphe cruciferarum* in camelina. However, in sole coriander plots, soilborne pathogens such as *Fusarium equiseti*, *Fusarium acuminatum*, *Epicoccum nigrum*, and *Epicoccum layuense* were 40% less prevalent compared to plots intercropped with camelina.

Image 15-22. The Major Weed Population Identified in the SCOOP trials



No chemical treatments were applied during these trials. The weeds identified in these untreated areas included *Chenopodium album* (common lambsquarters), *Orobanche* spp. (broomrape), *Cirsium arvense* (creeping thistle), *Sonchus oleraceus* (sow thistle), *Centaurea* spp., *Avena fatua* L. (wild oat), *Cuscuta* spp. (dodder), *Centaurea cyanus* (cornflower), *Convolvulus arvensis* (field bindweed), *Lactuca serriola* (prickly lettuce), *Xanthium strumarium* L. (Common Cocklebur), *Chenopodium albüm* (baconweed), Sisymbrium spp., and *Sinapis arvensis* (charlock mustard) (Figure 15-22).

2.6. Pest-Mediated Ecosystem Services

Early detection pest surveys commenced at early May, each plantation year. The plantation was inspected biweekly, spanning the early, flowering, and maturing phases throughout the growing season. Aphid species were generally identified as the primary infestors.

A total of forty plants were randomly chosen based on the plantation's size. Aphid-infested plant materials, including leaves, branches, and shoots, were collected, their coordinates were recorded, labeled before carrying them to the laboratory. The collected aphids were categorized, placed into tubes for macroscopic diagnosis, and subsequently stored. The aphid preparation processes followed the methodologies established by Hille Ris Lambers (1950) and Martin (1983).

Surveys were conducted using a simple random sampling technique, one of the probability sampling methods. These surveys aimed to represent the areas, ensuring that no less than 1% of the SCOOP planting area was included (Bora and Karaca, 1970). The identification process utilized by the reference of the insect collection of PPRI (Nazife Tuatay Plant Protection Museum).

According to the forementioned identification; the Coleoptera order *Phyllotreta nigripes* (Fabricus), *Phyllotreta corrugata* and Saulcy (Chrysomelidae), Lapsa sp. (Tenebrionidae), *Meligethes flavipes* (Nitidulidae), *Smaragdina biornata angorensis* (Chrysomelidae), Aphis fabae *Scopoli, Brevicoryne* brassicae (L.), Myzus (Nectarasiphon) persicae (Sulzer) belonging to the Hemiptera order were recorded at the experimantals.

Although the soil fleas (Phyllotreta spp.) were also detected no severe damages were obderved on the plant. A similar situation has been emphasized in studies conducted on this subject**. *C. sativa* was recorded relatively resistant to insect feeding and invasion** in previous studies (Nielsen et al. 2001; Gugel and Falk 2006; Francis and Warwick 2009). This resistance is thought to be caused by the absence of repellent stimulants or volatile phytochemicals that promote feeding (Henderson et al. 2004). They state that they do not feed even when they are densely populated on the plant, but that obligate feeding is observed under laboratory conditions when there are no different food sources (Pachagounder et al. 1998). Although colonization is observed on the Camelina plant in species belonging to the order Hemiptera, no damage, such as weakness or fumagine, was observed on the plants. Chesnais et al. (2015) investigated the colonization potential of 4 different aphid species on *C. sativa* under field conditions in France. In their studies, they stated that *Aphis fabae* (Scop) and *Myzus persicae* (Sulzer) lodged on the plant and were able to reproduce by feeding, and that they showed better colonization ability than other species, especially *Brevicoryne brassicae* (L.) and *Rhopalosiphum padi* (L.).

Although the Brassicaceae family is rich in insect diversity and abundance, the current mixed planting system limits this diversity. The number of species detected in camelina plots was lower in the mixed planting areas. As expected from research on medicinal and aromatic plants grown in intercropping systems, while reducing insect population and diversity, no damage was observed on the plant.

3. UNIBO's FINDINGS

3.1. Aims and scope

Analyzing the effect on above and below-ground biodiversity derived from the adoption of SCOOP intercropping in comparison to sole-crops under organic farming. At this scope UNIBO, in the field trials organized and reported with task 2.2 compared different combinations of underutilized crops sown in autumn and in spring with camelina.

3.2. Experimental lay out of the trials

Autumn sowing included camelina, pea and chickpea, while spring sowing included camelina, lentil and naked oat. Data for biodiversity assessments were collected during along three growing seasons for the autumn trials (2021-24) and only two growing seasons for the spring trials (2023-24). The surveyed parameters were: 1) weed presence at early stage (rosette stage for camelina, corresponding to BBCH stage 301), 2) pest and disease presence, 3) belowground biodiversity assessment.

For weed presence counting was carried out in replicated 20x20 cm squares positioned in each plot in the field, surveying the number of mono and dicot species present, as well as the total number of weeds. Early stage is definitely the most sensitive one for weed competition, and the adoption of intercropping is supposed to lower the weed pressure in comparison with the sole-cropping system.

For pest and disease presence the visual inspection of the plots was carried out regularly aiming at evaluating if the different cropping systems had different pest and disease pressure.

Finally, for belowground biodiversity, since it was not possible to ship soil samples to Turkey, as foreseen in the project proposal, UNIBO carried out these analyses internally in collaboration with the Microbiology Dept. Obviously, being this type of analyses very expensive and not budgeted we limited the study in only one combination of crops, camelina and pea, which seemed one of the more promising in terms of seed yield, in the field trials. Soil and rhizosphere samples were taken from all the plots before harvest and then analyzed to determine the differences in terms of on microbial communities, performing a β-diversity analysis of microorganisms.

Main results

3.3. Weed competition

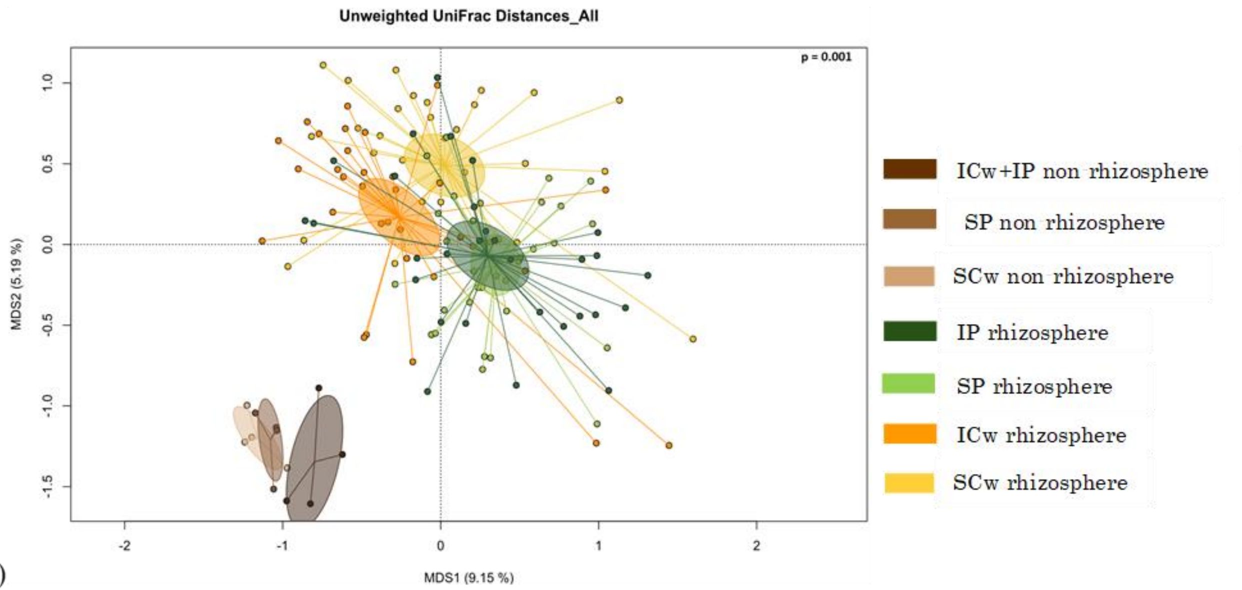
There were not significant differences in terms of weed presence, either monocot, dicot and total compared with the sole-crops in the autumn sown intercroppings. The weed presence was mainly driven by the dicot species which on average had triple the number of plants per m^2 compared to the monocot (18 vs. 6 species m^{-2}). The total number of weeds present was on average 24 species m^{-2}, ranging from 13 species m^{-2} in the sole-chickpea plots up to 31 species m^{-2} for sole-pea plots. When analyzing the weed presence in the spring intercropping systems, significant differences emerged between the systems, and intercropping appeared as good strategy for competing against weeds. Again, dicot species were more present than monocot species in the trial site. Significant differences emerged for the total number of weed species, with the intercropping systems, either camelina+lentil and camelina+naked oat, were able to control weed presence reporting a reduction of almost 70% of weed presence (10 vs. 26 species m-2, inter vs. sole-cropping system, $P \leq 0.05$).

3.4. Pest and disease presence

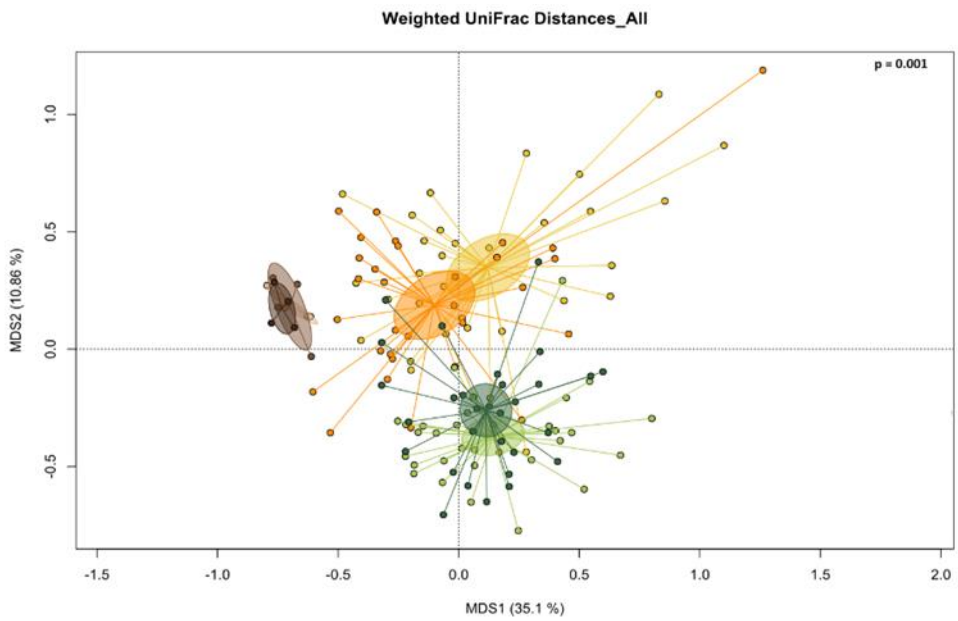
Cropping systems were not effective in controlling or reducing the presence of pests and diseases. Actually, the crops adopted in the SCOOP intercroppings were not very susceptible to biotic stresses, with only two exceptions: lentil that suffered in the first year from a strong attack of *Fusarium graminearum*, which was presumably already presented in the seeds used for establishing the trials (this was confirmed by lab analysis carried internally), and pea which was attacked by bean weevil (*Acanthoscelides obtectus*) just before harvest. Unfortunately for both abiotic stresses, the use of intercropping was not able to decrease the rate of infection.

3.5. Belowground biodiversity

Results refer to 2022-23 growing season, since the analysis for last year trials is still ongoing. Principal coordinates analysis (PCA) revealed a significant difference ($P \leq 0.01$) in the bacterial community compositions between non-rhizospheric and rhizospheric soils (Figure 8). Our findings indicate that intercropping impacts microbial composition differently across various ecological niches. Specifically, intercropping significantly altered the microbial composition of camelina in rhizospheric soil, while the different cropping systems don't differ in the non-rhizosphere soil.



A)



B)

Fig. 8. Principal Coordinates Analysis (PCoA) based on unweighted (A) and unweighted (B) UniFrac distances showing the variation of camelina and pea rhizosphere and non-rhizosphere soil microbiomes in the intercropping (ICw+ IP), sole-camelina (SCw) and sole-pea (SP).

The relative abundance of each soil microbial phylum (Fig. 9) in the intercropping was highly diverse compared to the sole-camelina samples but not compared to the sole-pea samples.

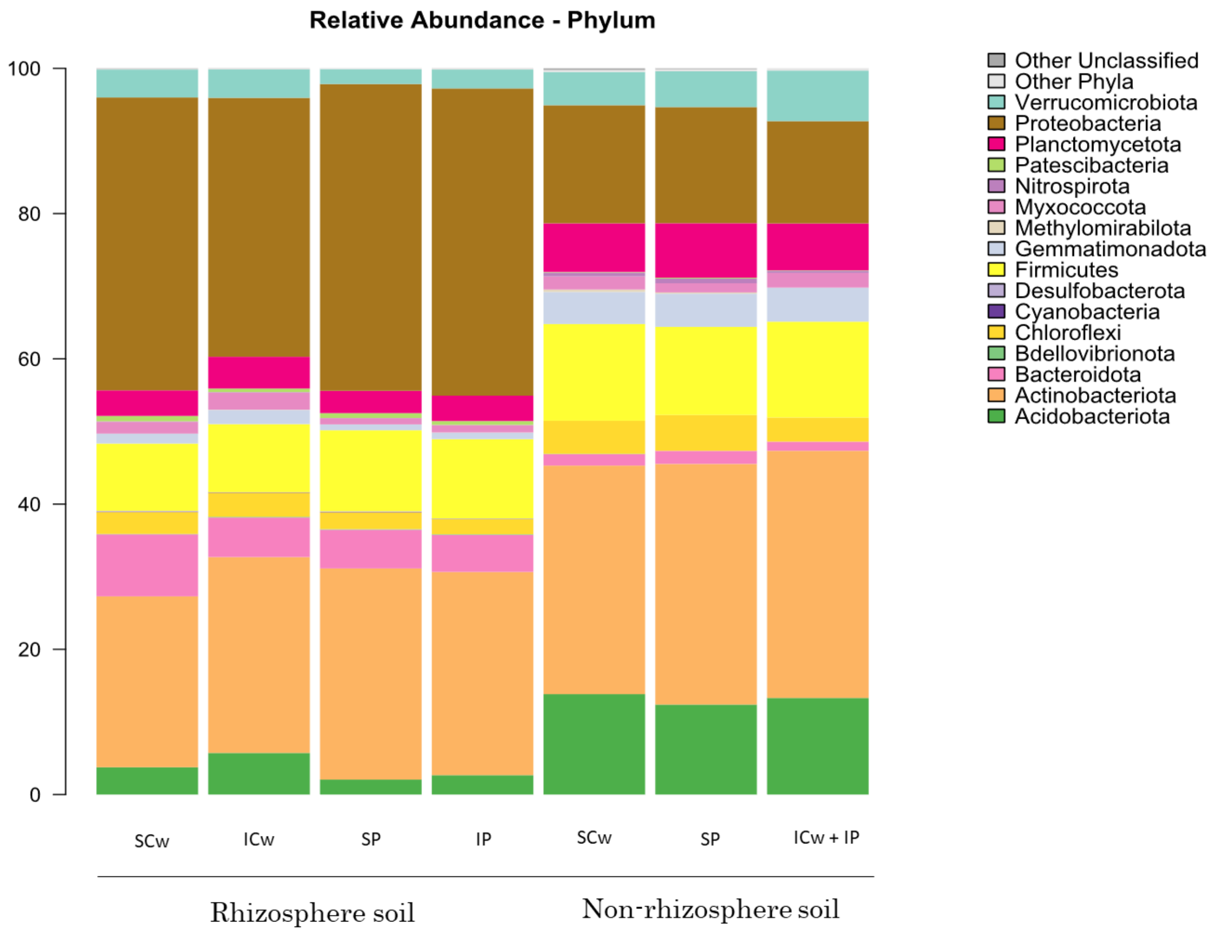


Fig. 9. Relative abundances from rhizosphere and non-rhizosphere soil bacterial communities of sole-camelina (SCw), sole-pea (SP), inter-camelina (IC), inter-pea (IP). The bacterial communities are clustered at the phylum level. Phyla that comprise 0.1% of a group were clustered in "Others." The size of each rectangle is proportional to the relative abundance of each group.

4. UWM's FINDINGS

4.1. Aims and scope

The aim of the study was to quantitatively and qualitatively analyse flora and entomofauna inhabiting organic cropping systems with camelina (*Camelina sativa*), lupine (*Lupinus angustifolius*), oat and spelt wheat, cultivated solely and with intercropping with camelina. Entomofauna analyses were made with particular emphasis on the most important groups of beneficial entomofauna (pollinating insects, predatory insects, ground beetles as zooindicators of habitat condition) and the most important plant pests and accompanying entomofauna.

4.2. Experimental lay out of the trials

The research was conducted in 2024 on the premises of an organic farm located in Butryny village (warminsko-mazurskie voivodship, Poland (N:53°36451,E: 20°33428). In this trial, camelina variety Olivia (0.30 ha), spelt wheat variety Wirtas (0.34 ha), oat (approx. 1 ha) (unknown variety) and narrow-leaved lupine (approx. 1 ha) (unknown variety) were sown as sole crops, and in the intercropping with camelina on areas of 0.52 ha (camelina+ spelt), 0.27 ha (camelina + oat) and 0.27 ha (camelina + lupine). Entomofauna studies were conducted from May 24 to July 23, 2024, on average every 10 days, catches and observations of beneficial entomofauna were conducted. In order to assess the entomofauna of the studied fields as accurately as possible, both beneficial and harmful, various research methods were used, such as observations, catches with an entomological net and catches in soil traps, each of which was dedicated to a specific group of studied insects. Monitoring of flying entomofauna, including pollinators, was carried out by inspecting each of the studied crops for 15 minutes each time. Individuals that were difficult to identify in the field were caught using an entomological net and taken to be identified in the laboratory.

Two research areas were designated on each of the 7 combinations, on which 4 soil traps were placed in transects to capture ground entomofauna. The traps were 400 ml plastic containers dug into the soil and filled to 1/3 of their volume with a preservative, which was an ethylene glycol solution. The traps were placed on May 24, 2024, and dismantled just before harvest - July 23, 2024. During this time, the contents of the traps were collected five times. The analysis of the material from the soil traps was made with the emphasis on zooindicators from the Carabidae family.

Harmful entomofauna was examined using an entomological scoop. Sampling consisted of 25 swings with an entomological net along a designated transect on each study plot. Additionally, plants were inspected to determine damage caused by pests.

The collected entomological material was processed in terms of taxonomic composition, abundance and selected biodiversity indices: Shannon (H'), Pielou evenness (J'), and Simpson species richness (D).

In the case of beneficial entomofauna, the species composition and abundance of the pollinators caught were determined. The values of Shannon species diversity index (H'), Pielou evenness (J') and Simpson diversity (D) were calculated. The share of the most numerous orders from the group of pollinating insects was shown.

In the study of predatory entomofauna, the focus was mainly on beetles from the family Coccinellidae and Carabidae. In the case of lady beetles, their species composition and the number of observed individuals and species were determined. Due to their small numbers and the number of species, diversity indices were not calculated for this group. The vast majority of predatory insects recorded in the studied fields were beetles from the family Carabidae, which are also bioindicators of the condition of habitats. Their species composition, abundance and number of species were determined in the individual study areas. The values of Shannon (H'), Pielou (J') and Simpson (D) indices were calculated. Due to the lack of normal distribution of data, the comparison of differences between means was performed using generalized linear models (GLM) at a significance level of $p>0.05$ in the Statistica 13.3 software. Anosim analysis was used to determine differences between Carabidae groups caught in crops. The comparison of the groups was visualized using NMDS analysis (non-metric multidimensional scaling) using PAST software.

Flora biodiversity in tested cropping systems was assessed during the silique development stage of camelina (June 2024) using 0.5 × 0.5 m frame, in 8 replicates on each field (sole sowings: lupin, spelt, oat, false flax; and intercroppings: camelina with lupin, spelt or oat). The results were recalculated per 1 m^2. In the laboratory, each sample (replicate) was analysed to determine the weed number and species composition. Weeds, after having their root systems removed, were weighed to determine their fresh mass, and then dried to determine their air-dry mass. In each sample, weeds of individual species were weighed separately. Weed nomenclature followed Mirek et al. [2002].

To determine plant biodiversity depending on the cultivation system, the Shannon diversity index (H') was used. To assess the evenness of species abundance distribution, Pielou's evenness index (J') was applied, and for assessing species dominance, Simpson's dominance index (D) was used.

4.3. Entomofauna results

As a result of the observations carried out, insects belonging to the following orders were recorded: springtails (Collembola), orthopterans (Orthoptera), hemiptera, lacewings (Neuroptera), beetles (Coleoptera), flies (Diptera), hymenoptera and butterflies (Lepidoptera).

4.3.1. Pollinators

In the conducted studies, pollinating insects belonging to four orders were recorded: beetles, flies, hymenoptera and butterflies (Table 1). The most numerous group of pollinators, both in terms of the number of individuals (Fig. 10) and the number of species (Fig. 11), were hymenoptera from the family Apiaridae, with the most numerous species such as the honeybee, the large earth bumblebee (*Bombus terrestris*) and the red-tailed bumblebee (*Bombus lapidarius*). Among the beetles, the monoceros beetle (*Notoxus monoceros*) deserves attention as a very effective pollinator, occurring particularly abundantly in oats and oat+camelina (Table 1, Fig. 10). An important group of pollinators from the order Diptera are insects from the family Syrphidae, which were recorded in large numbers in all the crops studied, both in sole cropping and in intercropping systems (Table 1).

Table 1. Species composition and number of pollinating insects recorded in the studied crops

Species	Cam+Lup	Cam+Spelt	Cam+Oat	Camelina	Lupin	Spelt	Oat
COLEOPTERA							
Notoxus monoceros	5	2	59	10	23	19	53
Oedemera virescens	1	0	0	1	0	0	0
Stenurella bifasciata	0	0	0	0	0	0	2
DIPTERA							
Syrphus ribesii	3	14	4	7	5	13	9
Episyrphus balteatus	24	20	18	13	16	15	12
Tachina fera	0	1	0	5	0	2	0
Bombylius posticus	0	0	1	0	1	0	0
HYMENOPTERA							
Sphecodes monilicornis	3	1	0	0	1	0	0
Chelostoma florisomne	2	2	0	0	0	0	0
Dasypoda hirtipes	0	0	0	1	2	1	0
Lassioglossum quadrinotatum	0	0	0	4	0	0	0
Lassioglossum calceatum	0	6	2	5	0	0	1
Andrena propinqua	0	3	0	1	3	4	0
Andrena flavipes	3	0	0	2	3	2	0
Apis mellifera	34	12	9	25	20	10	3
Halictus quadricinctus	4	3	0	0	0	0	0
Halictus subauratus	1	0	0	0	0	0	1
Halictus sexcinctus	0	0	0	0	0	1	0
Melitta leporina	0	1	0	1	0	0	0
Bombus campestris	0	0	0	0	1	0	0
Bombus lapidarius	8	1	3	11	14	1	1
Bombus hortorum	0	0	0	0	1	0	0
Bombus pascuorum	1	0	1	0	1	2	2
Bombus lucorum	3	1	0	0	0	0	0
Bombus terrestris	17	2	4	4	18	4	10
Hylaeus annularis	0	0	0	0	0	0	1
Panurgus calcaratus	0	3	0	0	0	0	0
Chrysis fulgida	2	0	0	0	0	2	0
Scolia hirta	0	0	0	0	1	0	0
LEPIDOPTERA							
Pieris napi	1	0	0	0	0	0	0
Pieris rapae	3	0	0	0	3	0	0
Pontia edusa	0	1	0	1	0	0	0
Zygaena filipendulae	0	0	0	0	0	1	0
Maniola jurtina	0	0	0	0	1	0	0
Colias hyale	1	0	0	0	1	0	0

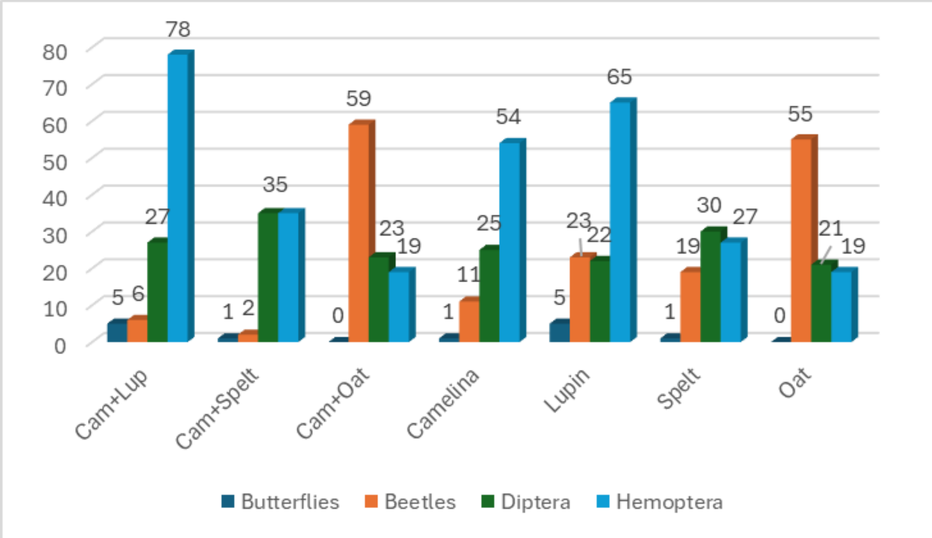


Figure 10. Share of pollinating insects from individual rows in the studied crops – quantitative aspect

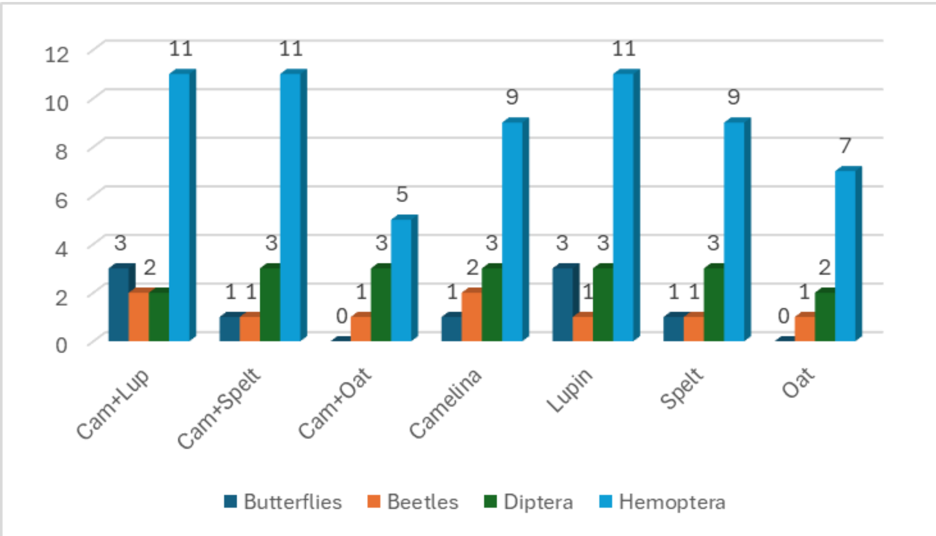


Figure 11. Share of pollinating insects from individual rows in the studied crops – qualitative aspect

Analyzing the entire group of pollinating insects, their highest numbers and species richness were found in lupine and camelina+lupine (Table 2), which is natural due to the nature of these plants, providing them with a food base as of pollen and nectar during flowering. The organic method of cultivation, which is associated with the occurrence of flowering weeds that are food for pollinators, influenced their higher diversity also in other crops, especially those intercropped with camelina (Table 2). Oat in sole cropping were characterized by the lowest value of the species diversity coefficient H' (0.66). Camelina and oat intercropping showed the highest value of Simpson's diversity index D, called the dominance concentration index (0.38), which was influenced by the high number of recorded individuals of one species of pollen-eating beetle - the monoceros beetle (Table 2, Table 1). The differences between oat and oat with camelina intercropping and other crops in terms of the occurrence of pollinating insects are also illustrated by the similarity dendrogram (Fig. 12).

Table 2. Number of individuals, species and diversity indices of pollinating insects collected in the studied fields

Index	Cam+Lup	Cam+Spelt	Cam+Oat	Camelina	Lupin	Spelt	Oat
Total Individuals	116	73	101	91	115	77	95
Mean Individuals	3.31	2.09	2.89	2.60	3.29	2.20	2.71
Total Species	18	16	9	15	18	14	11
Shannon H'	0.96	0.96	0.59	0.98	0.99	0.93	0.66
Evenness (Pielou J')	0.77	0.80	0.62	0.83	0.79	0.81	0.63
Diversity (Simpson D)	0.16	0.14	0.38	0.13	0.13	0.14	0.34

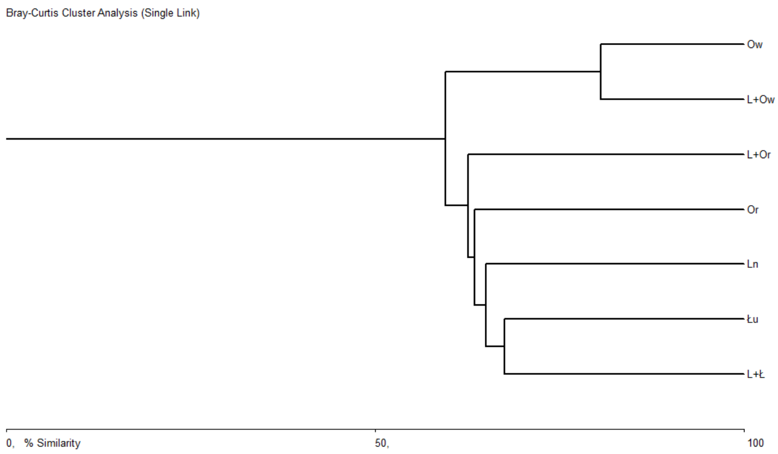


Figure 12. Diagram of similarity of pollinator clusters of the studied crops (Ow – oat, L – camelina, Or – spelt, Łu – lupin)

4.3.2.Predators

Studies indicate that the presence of predators in agrocenoses significantly reduces the need for chemical treatments, which not only reduces the costs of agricultural production, but also minimizes the negative impact on the environment. Both adult ladybugs and their larval forms were observed in the studied areas, which indicates a rich food base for these insects in the studied crops. The common ladybug was the most numerous, especially in oat cultivation. It was the least likely to choose camelina field (Table 3). The remaining species of ladybugs were recorded in the studied fields in smaller numbers (two-spotted ladybug) or as single individuals.

Table 3. Number of individuals and species of ladybirds found in the studied crops

Species	Cam+Lup	Cam+Spelt	Cam+Oat	Camelina	Lupin	Spelt	Oat
COLEOPTERA; occinelidae							
Coccinella septempunctata	32	16	40	9	21	21	51
Adalia bipunctata	21	9	7	2	7	10	4
Coccinella quinquepunctata	0	0	1	0	0	0	2
Psyllobora vigintiduopunctata	0	1	3	0	0	0	0
Propylea quatuordecimpunctata	0	0	1	0	0	0	0
Coccinula quatuordecimpustulata	0	0	0	0	1	0	0
Number of individuals	53	26	52	11	29	31	57
Number of species	2	3	5	2	3	2	3

4.3.3. Beneficial ground beetles (Carabidae) – as zooindicators indicating the condition of the studied habitats

Due to sensitivity to environmental changes, the presence, numbers and diversity of carabidae may indicate the degree of disturbance and biodiversity of agrocenoses. In field crops, carabidae also play an important role as natural predators, limiting the populations of pests such as aphids or moth larvae, which cause significant damage to crops. Therefore, they contribute to the biological protection of plants, which is of particular importance in the organic farming. As a result of the conducted research, 5668 individuals belonging to 51 species of Carabidae were caught during two months of observations (Table 4). Significant differences were observed in the number of species and individuals caught in individual crops ($p<0.001$).

Table 4. Number of individuals, species and diversity indices of Carabidae collected in the studied fields

Index	Cam+Lup	Cam+Spelt	Cam+Oat	Camelina	Lupin	Spelt	Oat
Total Individuals	613	699	1393	719	543	966	735
Mean Individuals	12.02	13.71	27.31	14.10	10.65	18.94	14.41
Total Species	28	23	31	28	26	29	31
Shannon H'	0.95	0.94	0.89	1.00	1.07	0.86	1.06
Evenness (Pielou J')	0.66	0.69	0.60	0.69	0.76	0.59	0.71
Domination (Simpson D)	0.16	0.17	0.22	0.13	0.12	0.22	0.12

Spelt cultivation was characterized by significantly higher species abundance (Fig. 4) and richness (Fig. 5) than the others, both in its sole and intercropping systems. Similar observations concern species diversity assessed using the Simpson index, which prefers abundant species, suggesting that spelt cultivation favors the occurrence of dominant carabid beetle species (Kromp, 1999; Thiele, 1977).

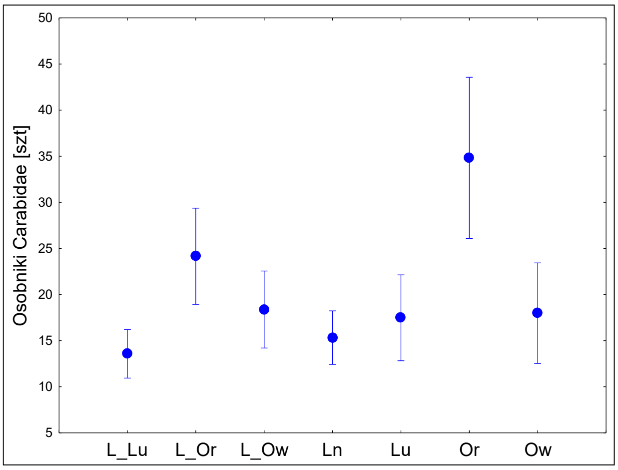


Figure 13-4. Average number of Carabidae individuals in the studied fields (Ow – oat, L – camelina, Or – spelt, Łu – lupin)

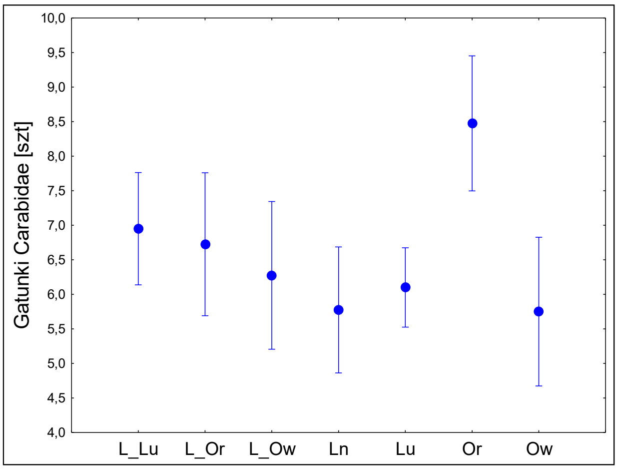


Figure 14-5. Average number of Carabidae species in the studied fields (Ow – oat, L – camelina, Or – spelt, Łu – lupin)

An interesting result is the high species diversity (Shannon H' and Pielou J' uniformity index) in the intercropping systems, especially in the intercropping of camelina with oat and lupine (Table 4). This may indicate a positive effect of the intercropping on species diversity, which is consistent with previous studies suggesting that intercropping can create more diverse microenvironments favorable to the species richness of ground beetles. According to studies by Kromp (1999) and Hummel et al. (2012) the number and diversity of these beetles increases compared to monocultures, which helps in biological control of pests such as aphids and fly larvae. It is worth noting the cultivation of lupine, in which, despite the lowest abundance and number of species, high species diversity of ground beetles was observed, especially in the intercropping systems. This suggests that camelina+lupine cultivation may create favourable environmental conditions for less numerous but diverse species of ground beetles, which is confirmed by the literature on the effect of diverse plant cover on Carabidae populations (Kromp, 1999, Dosdall et al., 2012, De Heij & Willenborg 2020).

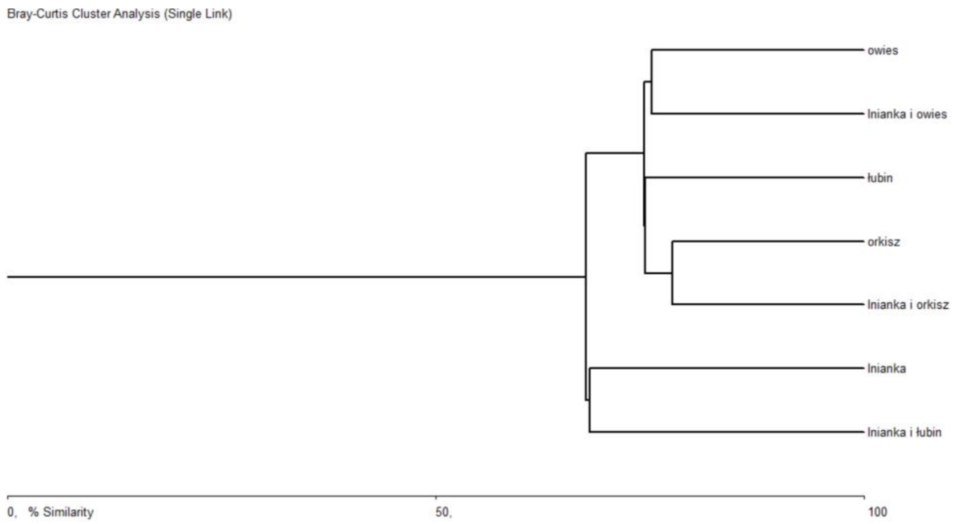


Figure 15-6. Dendrogram of similarities of Carabidae groups in the studied crops (Owies – oat, Lnianka – camelina, Orkisz – spelt, Łubin – lupin)

Cluster analyses confirm that the crop type is a key factor influencing the population structure of carabidae. Cereal crops such as spelt and oat showed high similarity (over 75%) to their counterparts in the intercropping system (Fig. 6). The results suggest that intercroppng have a limited effect on the composition of carabidae in these crops.

NMDS analysis also showed a clear separation of Carabidae assemblages depending on the crop type (Fig. 7). In particular, the carabidae assemblage of camelina crop differed the most from the others, suggesting specific habitat preferences of carabidae occurring in this crop species. Nevertheless, camelina intercropping with cereals did not cause significant changes in carabidae assemblages, which may indicate their limited effect on modifying the structure of Carabidae assemblages (Hummel et al., 2012; Magura & Lovei, 2021).

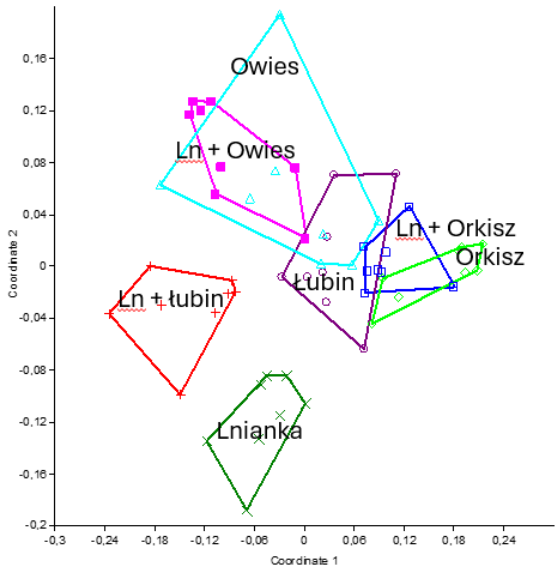


Figure 16-7. Non-metric multidimensional scaling (NMDS) diagram of Carabidae assemblages of the studied crops Owies – oat, Lnianka, Ln – camelina, Orkisz – spelt, Łubin – lupin)

4.3.4. Pests and accompanying entomofauna

The insects caught in camelina field belonged to both harmful species and accompanying entomofauna, not directly related to this plant. A total of 455 individuals were caught belonging to 4 orders: beetles (225 individuals), hemiptera (164), orthoptera (18) and diptera (48). Among the beetles, the common pollen beetle (119) was dominant, which is one of the most important pests of cruciferous plants, to which camelina belongs. In addition to the pollen beetle, the cabbage seedpod weevil (67) also occurred in large numbers, which is also one of the most important pests of cruciferous plants damaging generative parts. Pests typically associated with camelina, although occurring in low numbers, also include flea beetles.

A very numerous group inhabiting the camelina were hemiptera from the Cydnidae family. These are insects that have no economic significance in the cultivation of Brassicaceae plants, similarly to the caught individuals from the Rhyparochromidae family.

In the group of hemiptera, species characteristic of camelina were also caught, such as: *Eurydema ornatum*, rape bug, sloe bug, but they did not occur in numbers that could affect the condition of the crop (Table 6).

Table 6. Pests and accompanying entomofauna occurring in the cultivation of camelina as a sole crop

Species	Amount
Coleoptera	
Hoplia graminicola	3
Phyllotreta nemorum	8
Ceutorhynchus obstrictus	67
Meligethes aeneus	119
Alticini	6
Oulema melanopus	12
Silpha atrata	8
Oiceoptoma thoraci	2
Hemiptera	
Cydnidae	108
Rhyparochromidae	24
Dolycoris baccarum	13
Lygus rugulipennis	13
Notostira erratica	6
Eurydema ornatum	13
Eurydema oleraceum	12
Orthoptera	
Acrididae	18
Diptera	
Anthomyiidae	48

The analysis of the captured entomofauna in spelt sole cropping revealed the presence of insects belonging to four orders: beetles (69), hemiptera (150), orthoptera (173) and diptera (72). A total of 464 individuals were captured. The dominant species were insects belonging to the orthoptera, suborder Acrididae. However, this group does not pose a threat to cereal crops. Among the species classified as typical cereal pests, the following were found e.g.: the cereal flea beetle, the cereal leaf beetle, the blue leafhopper, the wheat leafhopper, *Cyphus pygmaeus*, tortoise bug, the cereal aphid and the bird cherry-cereal aphid. All of the above species occurred in small numbers. In the intercropping of camelina and spelt wheat, species characteristic of both plants were caught (302 individuals). When assessing the effect of camelina intercropping on the occurrence of spelt wheat pests, the absence of cereal aphids was observed. The number of other cereal pests was at a similar level as in the cultivation of sole spelt (Table 7).

Table 7. Entomofauna occurring in the cultivation of spelt wheat and camelina and spelt intercropping

Crop	Species	Amount
Spelt	**Coleoptera**	
	Phyllotreta vittula	31
	Phyllotretanemorum	7
	Oulema gallaeciana	5
	Oulema melanopus	11
	Ceutorhynchus obstrictus	6
	Hoplia graminicola	2
	Zabrus tenebrioides	3
	Oiceoptoma thoraci	4
	Hemiptera	
	Rhyparochromidae	8
	Cydnidae	3
	Cyphus pygmaeus	2
	Macrosteles leavis	11
	Dolycoris baccarum	12
	Eurygaster maura	19
	Lygus rugulipennis	21
	Notostira erratica	9
	Aelia acuminata	10
	Sitobion avenae	36
	Rhopalosiphum padi	19
	Orthoptera	
	Acrididae	173
	Diptera	
	Anthomyiidae	50
	Tipulidae	14
	Chlorops pumilionis	8
Spelt + camelina	**Coleoptera**	
	Phyllotreta vittula	14
	Phyllotreta nemorum	12
	Psylliodes chrysocephala	6
	Zabrus tenebrioides	7
	Ceutorhynchus obstrictus	43
	Ceutorhynchus rapae	6
	Meligethes aeneus	15
	Oulema melanopus	11
	Oiceoptoma thoraci	2
	Hemiptera	7
	Rhyparochromidae	13
	Macrosteles leavis	9
	Aelia acuminata	12
	Eurygaster maura	15
	Łygus rugulipennis	
	Orthoptera	92
	Acrididae	
	Diptera	38
	Anthomyiidae	

The entomofauna number in oat was quite low. A total of 164 individuals were caught, among which hemiptera (64) were dominant. Typical cereal pests such as: the cereal leaf beetle, the cereal flea beetle, leafhopper *Macrosteles leavis*, the bishop's mitre, the tortoise bug and the cereal aphid occurred in very small numbers. Similar results were also obtained from the camelina and oat intercropping (Table 8).

Table 8. Entomofauna occurring in oat and camelina+oat intercropping

Crop	Species	Amount
Oat	**Coleoptera**	
	Silpha atrata	1
	Hoplia graminicola	1
	Elateridae	8
	Oulema melanopus	11
	Phyllotreta vittula	8
	Oiceoptoma thoraci	11
	Pluskwiaki	
	Rhyparochromidae	3
	Cydcamenidae	8
	Macrosteles leavis	11
	Dolycoris baccarum	9
	Aelia acuminata	6
	Sitobion avenae	15
	Eurygaster maura	12
	Orthoptera	
	Acrididae	22
	Diptera	
	Anthomyiidae	38
Oat+camelina	**Coleoptera**	
	Silpha atrata	1
	Elateridae	5
	Phyllotreta vittula	4
	Zabrus tenebrioides	2
	Oiceoptoma thoraci	31
	Hemiptera	
	Rhyparochromidae	10
	Cynidae	36
	Macrosteles leavis	3
	Eurydema ornatum	3
	Lygus rugulipennis	7
	Eurygaster maura	5
	Aelia acuminata	3
	Dolycoris baccarum	8
	Notostira erratica	4
	Sitobion avenae	11
	Diptera	
	Anthomyiidae	36

In lupine sole cropping and camelina and lupin intercropping, the dominant species were weevils: *Charagmus gressorius* and *Charagmus griseus*. In sole lupin cultivation, they constituted 92% of the captured entomofauna, while in the intercropping, 66.25%. In both cases, the dominant species was *Ch. gressorius*, which constituted 68.5-75.3% of the weevil population. In these crops, alfalfa snout beetle, a pest of legumes, was also found. However, this species occurred at a very low number. The remaining insects constituted the accompanying entomofauna (Table 9).

Table 9. Entomofauna occurring in lupin and camelina+lupin intercropping

Crop	Species	Amount
Lupin	**Coleoptera**	
	Elateridae	5
	Phyllobius	7
	Hoplia graminicola	
	Charagmus gressorius	288
	Charagmus griseus	94
	Ceutorhynchus obstrictus	4
	Phyllopertha horticola	4
	Otiorhynchus ligustici	6
	Silpha atrata	6
	Oiceoptoma thoraci	1
	Hemiptera	
	Cynidae	62
	Rhyparochromidae	2
Camelina+lupin	**Coleoptera**	
	Phyllobius	4
	Otiorhynchus ligustici	3
	Phyllotreta nemorum	4
	Elateridae	1
	Hoplia graminicola	38
	Charagmus gressorius	148
	Charagmus griseus	68
	Oiceoptoma thoraci	4
	Hemiptera	
	Rhyparochromidae	10
	Cynidae	46

4.4. Flora biodiversity results

The highest number of species was determined in the cultivation of spelt wheat (27), and the lowest species diversity was found in the cultivation of oat (20) (Table 1). The highest number of weeds per 1 m2 was also found in the cultivation of spelt wheat. On the other hand, the lowest number of weeds was found in the intercropping of camelina and oat (195 plants). A similar, low value was obtained in the cultivation of oat alone (210 plants). The highest number of weeds (674 plants) was found in the cultivation of spelt in sole cropping (Table 1). In general, a tendency towards a lower number of weeds in intercropping compared to sole cropping was observed.

Table 10. Plant species, number of plants, number of species and total number of plants in individual cultivation systems per 1 m2 of field area.

Species/cropping system	cam+lupin	cam+oat	cam+spelt	camelina	lupin	oat	spelt
Achillea millefolium	1	1	1	1	1	0	1
Agropyron repens	10	3	10	5	24	11	12
Anchusa arvensis	7	10	3	3	20	0	4
Apera spica-venti	0	0	0	0	7	0	5
Arabidopsis thaliana	0	0	1	0	2	0	0
Avena fatua	76	11	0	2	71	7	4
Capsella bursa pastoris	0	1	1	0	0	1	4
Centaurea cyanus	28	10	5	1	72	24	2
Chenopodium album	28	5	7	9	6	1	11
Cirsium arvense	0	4	2	2	0	0	9
Equisetum arvense	4	3	2	3	0	0	1
Erodium cicutarium	1	0	0	0	2	0	0
Erysimum cheiranthoides	0	0	2	3	0	0	4
Galeopsis tetrahit	5	1	83	73	23	1	108
Galium aparine	0	0	0	0	0	1	1
Lapsana communis	1	0	0	0	0	1	0
Medicago sativa	0	0	0	0	0	1	0
Myosotis arvensis	1	0	0	0	3	0	0
Persicaria lapathifolia	2	8	263	165	38	10	236
Phacelia tanacetifolia	1	2	0	1	4	4	1
Poa annua	0	0	1	0	0	2	0
Polygonum aviculare	1	1	2	0	0	0	2
Polygonum convolvulus	72	104	126	132	80	125	197
Raphanus sativus	1	1	2	5	0	1	5
Rumex acetosella	0	0	1	1	0	0	4
Scleranthus annuus	43	19	19	16	89	20	4
Sonchus arvensis	1	2	2	0	1	0	2
Spergula arvensis	6	3	57	16	9	0	30
Stellaria media	0	1	0	0	0	0	0
Trifolium repens	0	0	3	1	0	0	10
Tripleurospermum maritimum	2	2	22	5	1	1	12
Veronica persica	0	6	0	0	0	1	0
Veronica arvensis	0	1	0	0	0	1	0
Vicia cracca	2	2	3	3	1	2	3
Vicia hirsuta	2	1	3	0	6	1	1
Viola arvensis	0	0	3	3	3	0	10
Total no. of plants	**289**	**195**	**617**	**447**	**458**	**210**	**674**
Total no. of species	**22**	**24**	**25**	**21**	**21**	**20**	**27**

A similar trend was observed for the weed mass, where their amount was lower in intercropping than in sole cropping systems (Table 2). The exception was found for oat in sole cropping, in which the lowest air-dry weed mass was found (71.4 g). The highest, however, was found in lupine cultivated alone (112.5 g), followed by spelt cultivated alone (109.1 g).

High biodiversity (H'>2) was characteristic of the camelina+lupin and lupin sole cropping fields. The other cultivation systems were characterised by moderate species diversity (in the range H'=1-2) (Table 3). On the other hand, spelt cultivation alone and camelina+spelt intercropping were characterised by low biodiversity (H'<1). Pielou's index (J') indicates high evenness (J'>0.6) of species occurring in the intercropping of camelina and lupin, as well as in lupin sole cropping (Table 3). The results for the dominance index indicate low dominance (camelina+lupin and lupin) and moderate dominance of one species (other cultivation systems). The dominant species were: *Avena fatua* (camelina + lupine), *Polygonum convolvulus* (camelina+ oats, oats), *Persicaria lapathifolia* (camelina+ spelt, camelina, spelt) and *Scleranthus annuus* (lupin) (Table 3).

Table 11. Fresh and air-dry weed mass (g) in individual cultivation systems per 1 m2 of field area

Cropping system	**Fresh mass of weeds (g/m^2)**	**Dry mass of weeds (g/m^2)**
camelina+lupin	209.7	67.2
camelina+oat	60.7	21.5
camelina +spelt	204.8	71.4
camelina	243.8	77.4
lupin	371.2	112.5
oat	52.2	22.5
spelt	343.0	109.1

Table 12. Results for biodiversity indices in individual cultivation systems

Biodiversity indicies/cropping system	**cam+lupin**	**cam+oat**	**cam+spelt**	**camelina**	**lupin**	**oat**	**spelt**
Shannon H'	2.06	1.91	1.80	1.75	2.28	1.55	1.88
Pielou (Evenness, J')	0.67	0.60	0.56	0.57	0.75	0.52	0.57
Domination (D)	0.17	0.30	0.25	0.25	0.13	0.38	0.24
Total numer of weeds	289	195	617	447	458	210	674
Amount of species	22	24	25	21	21	20	27
Dominant species	*Avena fatua*	*Polygonum convolvulus*	*Persicaria lapathifolia*	*Persicaria lapathifolia*	*Scleranthus annuus*	*Polygonum convolvulus*	*Persicaria lapathifolia*

4.5. Conclusions

In summary, the results of studies on zooindicators confirmed that the type of crop has a decisive influence on the numbers and species diversity of these beneficial insects. Diverse crops, such as combinations of cereals and oilseeds, e.g. camelina, create more complex microhabitats, which positively affects the species diversity of carabid beetles and translates into assistance in biological pest control.

The effect of camelina intercropping on pests of companion crops was not observed. However, it is difficult to clearly summarize the results because the species occurred in small numbers. Unfortunately, in the case of lupin, the results are not entirely reliable because it was grazed by wild animals during the vegetation period. Moreover, one-year entomological observations in organic field, infested with weeds, cultivated in next to sole croppings and intercroppings do not provide a basis for drawing conclusions on the effect of camelina intercropping on the occurrence of entomofauna in spelt wheat, oats and lupin.

The intercropping systems demonstrated beneficial effects in reducing weed biomass and maintaining higher biodiversity compared to most sole cropping systems. The lowest weed mass was recorded in oat sole cropping, while the highest occurred in lupin and spelt grown alone. Biodiversity indicators showed that camelina+lupin and lupin sole cropping supported greater species diversity and evenness with lower dominance of individual species. Dominant weed species varied depending on the cropping system, reflecting the influence of crop composition on weed community structure. It should be noted, however, that these results are based on a one-year experiment and should therefore be considered as preliminary data for further research on the effects of intercropping on weed occurrence and plant species biodiversity.

5. UP's FINDINGS

Evaluation of the impact on biodiversity in a certified organic field with sole crops of different varieties of camelina, pea and vetch and their intercropping were made according to the weed pressure, pest and diseases infestation and soil microbiota diversity.

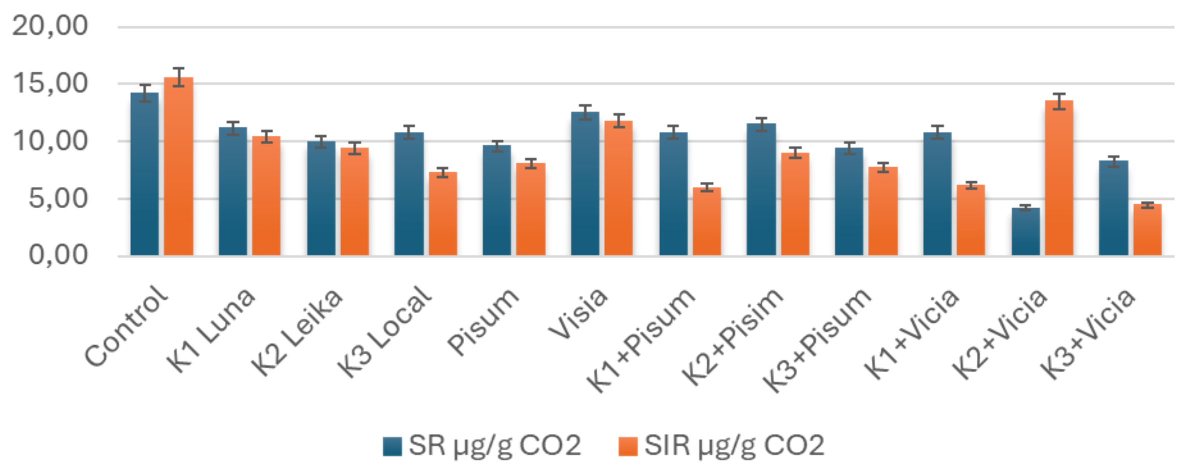
The phenological development of the crops and the present weeds, as well as their density and fresh weight were described in replicated 25 x 25 cm squares in each plot. Intercropping of camelina and both legume crops, sown in the spring, suppressed the development of grass weeds such as *Sorghum halepense*. It also formed lower fresh biomass weight in mixed plots. For the second important weed-*Setaria viridis,* the highest number of plants per m^2 was counted in the sole crops of pea and vetch. The lowest number of specimens was found in the intercropping system of the Bulgarian camelina landrace with vetch. The intercropping system of the well-developed Bulgarian camelina landrace with pea and vetch suppressed the development of the main grass weeds *Sorghum halepense and Setaria viridis.* The growth and development retardation are greater for the second weed.

The autumn sowing of the same trial shows faster development and better soil coverage for the local landrace of camelina, up to 80% in sole crops and almost the same in intercrop with vetch. Additionally, the legume covers up to 20% of the area which gives smallest chances for weed development related to the spring sowing.

Visual monitoring was carried out for pest and disease infestation. The intercropping system was found with lower population density of aphids as results of the biggest number of aphidophagues as *Coccinella septempunctata*, *Hippodamia variegata*, *Propylea quatuordecimpunctata*, *Harmonia axyridis* др.), larveas of *Scaeva pyrastri*, *Syrphus sp*., *Sphaerophoria sp*., imago and larvaes of *Chrysopa carnea*, *Chrysopa septempunctata* and *Chrysopa formosa*), some predatory molluscs of the family *Cantharidae* (*Cantharis fusca*, *Cantharis livida* and *Rhagonycha fulva*). Parasitic lice were also observed in colonies of the genus *Praon.* All the aforementioned aphidiophagous aphids, with their activities, have contributed significantly to reducing the population density and damaging activity of green pea aphid and black bean aphid in both cropping systems. Predatory and parasitic insects have been able to control aphids in mixed camelina crops, even during their periods of mass reproduction. Single plants of camelina were found with simptomatics of *Erysiphe cruciferarum* Opiz ex L. Junell and *Albugo candida* (Pers.) Kuntze. No significant differences were found in the pathogens in both cropping systems.

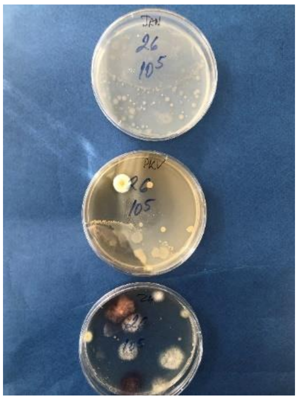
Intercropping of camelina with legumes (pea and vetch) create more favorable conditions for soil rhizosphere microbial communities, as evidenced by the measured higher intensity of soil respiration. At the same time, they have better carbon reduction abilities than sole crops of these species (Figure 17).

Figure 17. Soil rhizosphere microbial communities in intercropping of camelina with legumes (pea and vetch)

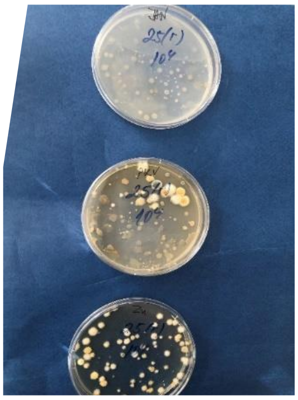


The analysis of nitrogen-fixing, phosphorus- and zinc-solubilizing microorganisms in soil from different trials showed the highest amount of nitrogen fixers in the variants with co-cultivation of camelina with legumes - camelina variety Lenka in combination with peas. In this combination, the highest number of P-solubilizing microorganisms was also found, while the lowest number was found in the Lenka sole crop. The trend was maintained for Zn-solubilizers. The fewest are in pure crop of a local landrace.

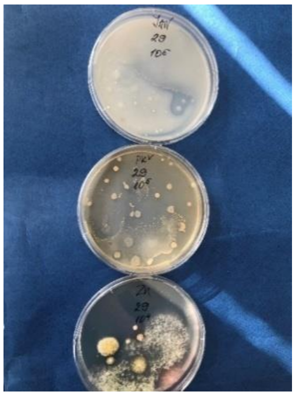
By screening for the gene encoding nitrogenase, 14 strains of nitrogen-fixing bacteria with the potential to promote plant growth, such as IAA production, phosphate dissolution, and siderophore production, were discovered. Thus, the newly isolated strains represent potentially excellent bioresources for future exploitation of functional genes and microbial inoculants.



23



24



25

Image 23-25. Isolation of nitrogen-fixing, phosphorus and zinc-degrading microorganisms

6. REFERENCES

Abrol D.P. 2012. Pollination Biology. Biodiversity Conservation and Agricultural Production. Springer. New York. Vol. 792.

Banaszak J., Cierzniak T. 2000. Ocena stopnia zagrożeń i możliwości ochrony owadów w agroekosystemach. Wiadomości Entomologiczne. 18 (2): 73-94.

Banaszak J., Izdebska B. 1994. Wpływ skażeń środowiskowych na pszczoły dziko żyjące, pszczołę miodną i produkty pszczele. Kosmos. 43 (2): 285-302.

Bora, T., Karaca, İ., 1970. Measurement of disease and damage in cultivated plants. Ege University Press, Bornova.

Chadfield VGA, Hartley SE, Redeker KR. Associational resistance through intercropping reduces yield losses to soil-borne pests and diseases. New Phytol. 2022 Sep;235(6):2393-2405. doi: 10.1111/nph.18302. Epub 2022 Jul 1. PMID: 35678712; PMCID: PMC9545407.

Chesnais Q., Verzeaux J., Couty A., Le Roux V., Ameline A. 2015. Is the oilseedcrop Camelina sativa a potential host for aphid pests? BioEnergy Res. 8:91–99. doi: 10.1007/s12155-014-9497-6.

Cong, W.-F., Hoffland, E., Li, L., Six, J., Sun, J.-H., Bao, X.-G., ... & van der Werf, W. (2015). Intercropping enhances soil carbon and nitrogen. Global Change Biology, 21(4), 1715-1726. https://doi.org/10.1111/gcb.12740

Dai Jing , Qiu Wei , Wang Nanqi , Wang Tianqi , Nakanishi Hiromi and Zuo Yuanmei 2019. From Leguminosae/Gramineae Intercropping Systems to See Benefits of Intercropping on Iron Nutrition. Frontiers in Plant Science V10. https://www.frontiersin.org/journals/plant-science/articles/10.3389/fpls.2019.00605. DOI=10.3389/fpls.2019.00605. ISSN=1664-462X

De Heij S.E, Willenborg C.J. 2020. Connected Carabids: Network Interactions and Their Impact on Biocontrol by Carabid Beetles. BioScience. 70 (6): 490-500

Dijkstra FA, Hobbie SE, Reich PB. 2006. Soil processes affectedby sixteen grassland species grown under different environ-mental conditions. Soil Sci Soc Am J 70(3):770–7 *(PDF) Biodiversity, Nitrogen Deposition, and CO2 Affect Grassland Soil Carbon Cycling but not Storage*. Available from: https://www.researchgate.net/publication/257469728_Biodiversity_Nitrogen_Deposition_and_CO2_Affect_Grassland_Soil_Carbon_Cycling_but_not_Storage [accessed Mar 25 2025].

Eyre M. D., Luff M. L., Leifert C. 2013. Crop, field boundary, productivity and disturbance influences on ground beetles (Coleoptera, Carabidae) in the agroecosystem. Agriculture, Ecosystems & Environment. 165: 60-67.

Francis A., Warwick S.I. 2009. The biology of Canadian weeds. 142. Camelina alyssum (Mill.) thell. C. microcarpa andrz. ex DC.; C. sativa (L.) crantz. Can. J. Plant Sci. 89:791–810. doi: 10.4141/CJPS08185

García-González, M., et al. (2016). Effects of arbuscular mycorrhizal fungi on the growth and phosphorus uptake of Camelina sativa under different levels of phosphorus supply. Journal of Plant Nutrition, 39(12), 1806-1816.

Gugel R.K., Falk K.C. 2006. Agronomic and seed quality evaluation of Camelina sativa in western Canada. Can. J. Plant Sci. 86:1047–1058. doi: 10.4141/P04-081.

Henderson A.E., Hallett R.H., Soroka J.J. 2004. Prefeeding behavior of the crucifer flea beetle, Phyllotreta cruciferae, on host and nonhost crucifers. J. Insect Behav. ;17:17–39. doi: 10.1023/B:JOIR.0000025130.20327.1a.

Holland J. M., Luff M. L. 2000. The effects of agricultural practices on Carabidae in temperate agroecosystems. Integrated pest management reviews. 5 (2): 109-129.

Holland J. M., Thomas C. F. G., Birkett T., Southway S., Oaten H. 2005. Farm-scale spatiotemporal dynamics of predatory beetles in arable crops. Journal of applied ecology. 42 (6): 1140–1152.

Jenkinson, D. S., & Ladd, J. N. (1981). Microbial biomass in soil: Measurement and turnover. Soil Biochemistry, 5, 415-471.

Koivula M. 2011. Useful model organisms, indicators, or both? Ground beetles (Coleoptera, Carabidae) reflecting environmental conditions. ZooKeys. 100: 287–317.

Kosewska A., Nietupski M., Ciepielewska D., Słomka W. 2009. Czynniki wpływające na struktury zgrupowań naziemnych biegaczowatych (Col., Carabidae) w wybranych uprawach zbóż. Progress in Plant Protection. 49 (3): 1035-1046.

Kotze D. J., Brandmayr P., Casale A., Dauffey-Richard E., Dekoninck W., Koivula M., Lövei G., Mossakowski D., Noordijk J., Paarmann W., Pizzolotto R., Saska P., Schwerk A., Serrano, J., Szyszko J., Taboada A., Turin H., Venn S., Vermeulen R., Zetto-Brandmayr T. 2011. Forty years of carabid beetle research in Europe – from taxonomy, biology, ecology and population studies to bioindication, habitat assessment and conservation. ZooKeys. 100: 55–148.

Kromp B. 1999. Carabid beetles in sustainable agriculture: a review on pest control efficacy, cultivation impacts and enhancement. Agriculture, Ecosystems & Environment, 74, 187-228.

Lehmann, A., & Rillig, M. C. (2015). Arbuscular mycorrhizal contribution to copper, manganese and iron nutrient concentrations in crops – a meta-analysis. Soil Biology and Biochemistry, 81, 147-158.

Lövei G. L., Sunderland K. D. 1996. Ecology and behavior of ground beetles (Coleoptera: ground beetleae). Annual Review of Entomology. 41: 231–256.

Mirek Z., Piękoś-Mirkowa H., Zając M.: Flowering plants and Pteridophytes of Poland – a checklist. – W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków, 2002, ss. 442.

Muyzer, G., et al. (1993). Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Applied and Environmental Microbiology, 59(3), 695-700.

Nielsen J.K., Hansen M.L., Agerbirk N., Petersen B.L., Halkier B.A. 2001. Responses of the flea beetles Phyllotreta nemorum and P. cruciferae to metabolically engineered Arabidopsis thaliana with an altered glucosinolate profile. Chemoecology. 11:75–83. doi: 10.1007/PL00001835.

Pachagounder P., Lamb R.J., Bodnaryk R.P. 1998. Resistance to the flea beetle Phyllotreta cruciferae (Coleoptera: Chrysomelidae) in false flax, Camelina sativa (Brassicaceae) Can. Entomol. 130:235–240. doi: 10.4039/Ent130235-2.

Pruszyński G. 2008. Chrząszcze – Biedronkowate. W: Tomalak M., Sosnowska D. (red). Organizmy pożyteczne w środowisku rolniczym. IOR Poznań: 46-49.

Rader R, Bartomeus I, Garibaldi LA I in. 2016 Non-bee insects are important contributors to global crop pollination. PNAS 113:146-51.

Rainio J., Niemelä J. 2003. Ground beetles (Coleoptera: Carabidae) as bioindicators. Biodiversity & Conservation. 12: 487-506.

Redecker, D., et al. (2003). Molecular identification of arbuscular mycorrhizal fungi in roots: perspectives and problems. Fungal Diversity, 33, 123-144.

Séguin-Swartz, G., Eynck, C., Gugel, R. K., Strelkov, S. E., Olivier, C. Y., Li, J. L., … Falk, K. C. (2009). Diseases of Camelina sativa(false flax). Canadian Journal of Plant Pathology, 31(4), 375–386. doi:10.1080/07060660909507612

Smith, S. E., & Read, D. J. (2008). Mycorrhizal Symbiosis. Academic Press.

Sollai G., Solari P. 2022. An overview of "Insect Biodiversity". Diversity. 14 (2): 134.

Thiele H. U. 1977. Carabid beetles in their environments. A study on habitat selection by adaptations in physiology and behavior. Springer – Verlag, Berlin, 328 pp.

Verbruggen, E., et al. (2012). Mycorrhizal fungal diversity and productivity are reduced by intensive agricultural practices in a long-term field experiment. Ecology Letters, 15(9), 972-981.

Verma, P.R., and Saharan, G.S. (1994). Monograph on alternariadiseases of crucifers. Minister of Supply and Services Canada,Ottawa, Ont. Agric. Can. Res. Br. Tech. Bull. 1994-6E.

Zhang, X., Wu, Y., Liu, S., Li, J., Jiang, Z., Luo, H., & Huang, X. (2024). Plant growth and development of tropical seagrass determined rhizodeposition and its related microbial community. *Marine Pollution Bulletin*, *199*, 115940. https://doi.org/https://doi.org/10.1016/j.marpolbul.2023.115940

Zubek, S., et al. (2010). The influence of arbuscular mycorrhizae on plant growth and nutrient uptake in medicinal plants grown under organic farming conditions. Agriculture, Ecosystems & Environment, 137(1-2), 159-167.