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Effects of crop diversification on weed communities and crop performance in organic arable systems in Southern Sweden

Effetti della diversificazione colturale sulle comunità infestanti e sulle prestazioni delle colture nei sistemi seminativi agricoli biologici nella Svezia meridionale

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Abstract

Organic farming relies mostly on crop diversification and ecological processes to manage weeds. However, perennial weeds remain a major problem, especially due to their persistence and the limited weed management techniques used in organic systems. This thesis investigates how crop diversification through intercropping and multispecies cover crops affects weed communities, crop-weed interactions, and crop performance within two crop rotation systems in southern Sweden. The study was carried out within the Diversification through Rotation, Intercropping, and Multicropping (DRIM) experimental trial at the Alnarp SITES Lönnstorp Research Station. A Reference rotation and a Diversified rotation were compared across 48 plots arranged in a randomized complete block design. Abundance and composition of weeds was recorded and analyzed together with perennial-thistle abundance, soil microclimate, fractional green canopy cover, leaf chlorophyll, crop and weed biomass and grain yield.

In the experiment, a total of 1435 weeds from 16 taxa were recorded. Weed communities differed more among individual crop treatments than between the two rotation systems, which had similar levels of overall weed abundance and perennial thistles. The oat-lupin treatment had a higher total weed abundance but slightly fewer perennial thistles, while the pea-barley treatment had fewer weeds overall but greater dominance of perennial thistles. This shows that a lower number of weeds does not necessarily mean better weed management, since some crop combinations may favor particular annual or perennial weed groups. No significant differences between the rotation systems were found for soil microclimate, fractional green canopy cover, or leaf chlorophyll. In exploratory analyses based on system × crop-treatment means aggregated across blocks, crop biomass was negatively associated with weed biomass and positively associated with grain yield. These descriptive relationships were consistent with the third hypothesis but do not demonstrate cause-and-effect relationships because the data were aggregated across blocks.

Overall, the results suggest that the success of diversification in organic cropping systems depends on more than simply increasing the number of crop species in the rotation. Crop competitiveness, canopy development, seeding density, and the effectiveness of specific crop combinations in controlling perennial weeds play a crucial role. Given the limited observation period of this thesis, long-term field experiments are needed to validate whether these observed patterns remain

consistent across successive rotation cycles and different seasonal conditions, especially with respect to perennial-thistle dominance and crop-specific performance.

Riassunto

L'agricoltura biologica si basa ampiamente sulla diversificazione colturale e sui processi ecologici per la gestione delle infestanti. Tuttavia, le infestanti perenni rappresentano ancora un problema rilevante, soprattutto a causa della loro persistenza e delle limitate tecniche di gestione delle infestanti disponibili nei sistemi biologici. La presente tesi analizza l'effetto della diversificazione, attraverso consociazioni e colture di copertura multispecifiche, sulle comunità infestanti, sulle interazioni tra colture e infestanti e sulle prestazioni produttive delle colture in due sistemi di avvicendamento colturale nel sud della Svezia. Lo studio è stato condotto nell'ambito della prova sperimentale *Diversification through Rotation, Intercropping, and Multicropping* (DRIM), presso la stazione di ricerca Alnarp SITES Lönnstorp. Sono stati confrontati un avvicendamento di riferimento (*Reference rotation*) e un avvicendamento diversificato (*Diversified rotation*) in 48 parcelle disposte secondo un disegno a blocchi randomizzati completi. L'abbondanza e la composizione delle infestanti sono state rilevate e analizzate insieme all'abbondanza del cardo perenne, al microclima del suolo, alla copertura verde frazionaria della chioma, al contenuto di clorofilla fogliare, alla biomassa delle colture e delle infestanti e alla resa in granella.

Nel complesso della prova sono state registrate 1.435 infestanti appartenenti a 16 taxa. La composizione delle comunità infestanti ha mostrato differenze maggiori tra i singoli trattamenti colturali che tra i due sistemi di avvicendamento, i quali hanno evidenziato livelli simili di abbondanza complessiva delle infestanti e di presenza del cardo perenne. La consociazione avena–lupino ha presentato una maggiore abbondanza complessiva di infestanti, ma una presenza leggermente inferiore di cardo perenne, mentre la consociazione pisello–orzo ha mostrato una minore abbondanza complessiva di infestanti, ma una maggiore dominanza del cardo perenne. Ciò evidenzia come una minore abbondanza complessiva di infestanti non corrisponda necessariamente a una migliore gestione delle infestanti, poiché alcune combinazioni colturali possono favorire specifici gruppi di infestanti annuali o perenni. Non sono state rilevate differenze significative tra i sistemi di rotazione per quanto riguarda il microclima del suolo, la copertura della chioma verde o il contenuto di clorofilla fogliare. Nelle analisi esplorative basate sulle medie relative alla combinazione sistema × trattamento colturale, aggregate a livello dei blocchi, la

biomassa della coltura è risultata negativamente associata alla biomassa delle infestanti e positivamente associata alla resa in granella. Tali relazioni descrittive erano coerenti con la terza ipotesi, ma non dimostrano rapporti di causa-effetto, poiché i dati erano aggregati a livello dei blocchi.

Nel complesso, i risultati suggeriscono che il successo della diversificazione nei sistemi di coltivazione biologica dipende da fattori che vanno oltre il semplice aumento del numero di specie incluse nell'avvicendamento colturale. La competitività delle colture, lo sviluppo della copertura della chioma, la densità di semina e la capacità di specifiche combinazioni colturali di sopprimere le infestanti perenni rappresentano anch'essi fattori importanti. Poiché il presente lavoro di tesi ha considerato un periodo di osservazione limitato, sono necessari esperimenti di campo di più lunga durata per determinare se i modelli osservati rimangano costanti nel corso di successivi cicli di avvicendamento e in condizioni stagionali differenti, in particolare per quanto riguarda la dominanza del cardo perenne e le prestazioni delle singole colture.

1. INTRODUCTION

1.1 Organic farming

Organic farming is a key approach to sustainable agriculture because it aims to produce food while protecting soil, water, biodiversity, and other ecosystem services through ecological processes rather than synthetic fertilizers and pesticides (Rigby & Cáceres, 2001; Birkhofer et al., 2016). Crop rotation, cover crops, organic amendments, and biological regulation can improve soil fertility, support beneficial organisms, and reduce dependence on external chemical inputs (Barbieri et al., 2017; Krause et al., 2024). These advantages, however, also create a major management challenge: without selective herbicides, weeds must be controlled through crop competition, cultural practices, and mechanical operations.

Additionally, organic agriculture can experience lower yields during transition and higher labor requirements for weed control, particularly when perennial weeds become established (Kirchmann et al., 2007; Bond & Grundy, 2001). For perennial weeds, whose persistent underground roots allow them to survive disturbance, regenerate from fragments, and compete with crops over several seasons, weed control is quite a challenge. Understanding how crop diversification influences these weeds is therefore essential for developing organic systems that are both productive and environmentally sustainable.

1.1.2 Weed Control in organic farming

Conventional farmers usually control weeds by applying synthetic herbicides regularly. This approach works for a while, but it has caused issues such as herbicide-resistant weeds and water pollution (Liebman and Davis, 2000). Since organic farmers cannot use these chemicals, they depend on a combination of mechanical, biological, and cultural methods (Hatcher and Melander, 2003).

In organic farming, non-chemical weed control can be expensive and may require a lot of labor (Dhakal et al., 2024). Instead of simply spraying herbicides, organic farmers must plan ahead and use different strategies, starting before planting. This involves planning crop rotations, choosing cover crops carefully, and timing mechanical weed control well (Melander et al., 2005). How well these methods work depends on the types of weeds and the local conditions (Bond and Grundy, 2001).

Weed challenges are more common in organic systems, and if weeds are not managed well, crop yields can fall sharply (Adeux et al., 2019). Weeds mostly compete with crops for light, water, and nutrients (Ryan et al., 2009). They can also attract insects and birds that spread diseases (Marshall et al., 2003). In addition, weeds can make harvesting more difficult and lead to grain contamination and higher drying costs.

1.1.3 The growing challenge of Perennial Weeds

Perennial weeds are much more difficult to control in organic systems than annual weeds, which are often managed with tillage and mechanical methods (Lundkvist and Verwijst, 2011). For instance, *Sonchus arvensis* L. (field sow thistle) and *Cirsium arvense* (L.) Scop. (Canada thistle) have large underground root systems and can regrow from even small root fragments (Anbari et al., 2016a).

Reduced-tillage methods, which are widely used in organic farming to improve soil and store carbon, have unintentionally helped these stubborn weeds grow (Melander et al., 2005). Perennial weeds store energy in their deep roots, which are usually not affected by reduced tillage (Vanhala et al., 2006). When tillage is used, it often breaks up the roots rather than removing them, making it easier for the weeds to spread (Ringselle et al., 2018).

Persistent perennial weeds present a significant economic challenge due to their extensive underground systems. This challenge is particularly pressing in northern European organic systems, where, like in any organic system, herbicide use is limited. In such contexts, weed control relies heavily on crop competition, crop rotation, cover crops, and mechanical management. Studies previously carried out on the same experimental site in Alnarp lay the essential groundwork for this research. Helgesson (2019) analyzed the weed seed bank in a pea-barley intercropping system, as well as in sole pea, sole oat, and black fallow systems. Additionally, Uduwalage (2024) investigated the diversity and composition of weed seed banks in Reference and Diversified rotations. Both studies concluded that there was limited evidence of short-term effects from the treatments on seed-bank characteristics, emphasizing the need to supplement seed-bank assessments with in-field observations of standing weed communities and crop performance.

Since weeds cannot be managed through routine applications of selective herbicides in organic systems, farmers must intentionally alter the growing environment so that crops are able to compete successfully. The following background therefore moves from the general mechanisms of crop-weed competition to the management role of crop diversification, before focusing on the biology and control of the perennial weeds examined in this study.

1.2 Competition in Agroecosystems

Competition is a fundamental ecological interaction in which two or more organisms strive for the same scarce resources needed to survive, grow, and reproduce (Savić et al., 2025). In agroecosystems, competition is mainly between crops and spontaneous weed populations for basic inputs such as sunlight, water, and soil nutrients (Ryan et al., 2009). The result of this interaction is a key determinant of crop yield, quality, and the overall economic viability of the agricultural system.

The intensity of competition is governed by several factors, including the density and distribution of both crops and weeds, the timing of their emergence relative to each other, and their respective growth rates and morphological traits (Savić et al., 2025). In organic systems, where synthetic inputs are prohibited, this ecological dynamic is particularly important, since weed control depends on the competitive power of the crop and on strategic use of cultural practices, for instance, crop rotation, selection of competitive crops, optimized sowing density, cover crops, and mechanical weed control, to shift the competition in favor of the cultivated species.

1.2.1 Competition for Light

Competition for light often starts at the beginning of the growing season and is the main driver of biomass accumulation and yield potential. Light is a unique agricultural resource because, unlike water or nutrients, it cannot be stored in the soil for later use; it must be captured in the canopy of plants on the spot (Savić et al., 2025).

Weeds that germinate simultaneously with or before the harvest, or which have rapid early growth and high branching growth, may rapidly rise above the crop canopy. This results in direct shading and reduces the amount of photosynthetically active radiation (PAR) reaching the lower leaves of the crop. For example, *Sonchus arvensis* L., a perennial weed known for its ability to produce a high, thick canopy, can shade spring cereals considerably, especially in the sensitive early growing stages (Anbari et al., 2016b).

The effect of shading is not just to reduce photosynthesis. It also triggers physiological responses in plants, such as the shade avoidance response (Swanton et al., 2026). When plants detect a reduction in the ratio of red to far-red light (signaling that they are being shadowed), they may favor the growth of the stem at the expense of root development and reproduction. This may lead to spindly, weak crop plants, which are more susceptible to lodging and reduced grain filling capacity.

Recent research has shown that the interaction of crops and weeds in terms of light is more complex than simple shading. Even before direct shading occurs, plants can detect the presence of their neighbors by changing the spectrum of light reflected off neighboring leaves (Horvath et al., 2023). This early detection may result in physiological changes in the crop that reduce its yield potential long before the weed becomes physically dominant. In organic systems, managing crop distribution and selecting competitive varieties with fast canopy closure represent key strategies for decreasing these light losses.

1.2.2 Competition for Water

Water availability is a major constraint on crop productivity in many regions, and weeds can be a challenging competitor for this important resource. The ability of a plant to compete for water is largely determined by the depth, extent, and density of its root system (Savić et al., 2025).

The extensive and deep root systems of perennial weeds, such as *Sonchus arvensis* L. and *Cirsium arvense* (L.) Scop., usually surpass those of annual crops. For example, *Sonchus arvensis* L. can grow roots that reach deep into the soil profile, giving it access to water reserves that annual crops with shallower roots cannot reach (Anbari et al., 2016). During times of drought or during crucial stages of crop growth and grain filling, this characteristic can be especially destructive.

The timing of weed growth also affects competition for water. Weeds that establish their root systems at the beginning of the season may reduce soil moisture content, which may lead to water stress in the developing crop. This stress may be manifested by reduced leaf expansion, reduced stomatal flux, and ultimately reduced biomass accumulation (Savić et al., 2025).

In the field, we can monitor the effect of weed competition on water availability by measuring the volume of water content (VWC). The significant reduction of VWC near weed patches compared to weed-free areas is a clear indication of the competitive pressure exerted by the weed population (Robinson et al., 2008).

1.2.3 Competition for Nutrients

Competition for soil nutrients is a key issue in organic farming, where nutrient availability is often more fluctuating and potentially more limited than in conventional systems (Karlsson et al., 2025). Both crops and weeds need macronutrients such as nitrogen (N), phosphorus (P), and potassium (K) as well as other micronutrients to grow and develop.

Weeds are often very effective at removing nutrients from the soil, even in low concentrations. Many species have developed rapid uptake kinetics, which allows them to consume available nutrient reservoirs before the crop can access them (Savić et al., 2025). *Sonchus arvensis* L., for instance, is recognized as a heavy feeder, especially of nitrogen, which is often the most limiting nutrient in organic systems (Lundkvist and Verwijst, 2011). The interaction between fertilization and weed competition is complex. The addition of organic amendments is intended to increase crop yields, but may also unintentionally encourage weed growth. If the timing and application of fertilizers are not effectively managed, weeds may benefit more from the added nutrients than the crop, resulting in increased weed biomass and consequently reduced crop yield (Karlsson et al., 2025).

The nitrogen status of the plant is closely related to its chlorophyll content. Non-destructive chlorophyll measurements, such as the chlorophyll index (CCI), may serve as a reliable proxy for plant health (Fitzgerald et al., 2010). A decrease in CCI among crop plants growing near perennial weed patches often indicates that the weed is successfully competing with the crop for the available nitrogen in the soil.

1.2.4 The Chemical aspect of Competition (Allelopathy)

In addition to direct competition for resources, many weed species exert influence on their neighbors through allelopathy, the release of biochemical substances into the environment that inhibit the growth and development of other plants (Savić et al., 2025). Allelopathic interactions may occur in many ways, such as leaching of chemicals from leaves, exudation from roots, and degradation of plant residues in soils. Although the allelopathic effects of *Sonchus arvensis* L. and *Cirsium arvense* (L.) Scop. are not as well studied as their competitive capacity in resource competition, it has been shown that they can produce compounds that adversely affect the germination and early growth of certain crops (Anbari et al., 2016a). This chemical interference adds a degree of complexity to the dynamics of weed growth in organic systems.

1.2.5 The Critical Period for Weed Control (CPWC)

To develop efficient management strategies, it is important to understand the timing of competition. The Critical Period for Weed Control (CPWC) is a concept in weed science, defined as the period during the crop growth cycle when weeds must be controlled to avoid significant yield losses (Savić et al., 2025).

The CPWC is divided into two phases:

The beginning of the critical period: the point at which weed competition begins to cause a significant decrease in the crop's yield.

End of the critical period: A point after which the weeds that have emerged no longer have a significant impact on the final yield.

Knowing the CPWC enables farmers to time their weeding operations more accurately, lowering labor costs and minimizing the environmental impact of mechanical controls (Melander et al., 2017). However, the life cycle of perennial weeds makes this concept difficult, as they may appear and reappear throughout the season and often require multiple measures to keep them below economic thresholds.

1.3 Perennial Weed Management in Organic Systems

Organic farming systems are identified by their reliance on ecological processes and the combining of a variety of management practices for sustaining productivity and pest control (Altieri, 2019). The absence of synthetic herbicides in these systems leads to a much different weed behavior than in conventional farming. In organic systems, the weed flora is usually more diverse, and the interaction between crops and weeds is more complex and biologically driven (Mwangi et al., 2024). The rest of this section analyzes why diversified management solutions are needed, looks at crop rotation, cover crops and intercropping, and discusses the problems of perennial weeds in northern Europe and the tactics used to tackle them.

Liebman and Gallandt (1997) suggest that the effectiveness of weed control through crop diversification lies in the concept of the "many little hammers". This approach involves combining several 'weak' weed-suppressive strategies for strong and long-lasting weed control, which is especially useful in organic systems.

Each diversification strategy (crop rotation, cover crops, intercropping) represents a unique "hammer" that targets weeds at different stages of their life cycle and through different ecological mechanisms (Liebman and Gallandt, 1997). For instance, an intercrop increases resource competition, a subsequent cover crop offers allelopathic effects and competitive suppression, and a varied crop rotation may disturb a particular weed's life cycle (Liebman and Gallandt, 1997). The cumulative effects of numerous non-lethal stresses, which can be far greater than the sum of their individual parts, could reduce weed pressure significantly over time (Liebman and Gallandt, 1997).

Farmers can increase the agroecosystem's resistance to weed shifts or adaptations by using a variety of weed management tools, consequently reducing their dependence on any single technique. This prevents weeds from developing resistance or adjusting to a single pressure, which is often the case in systems that are simplified and input intensive.

In the long-term, diverse cropping systems typically promote weed communities that are more stable and less troublesome. Instead of completely eradicating weeds, the goal is to control them to the point where they do not cause economic losses while conserving a varied weed flora that can support ecosystem services. In contrast, traditional methods frequently result in the dominance of a small number of extremely troublesome, herbicide-resistant species.

The "many little hammers" strategy works especially well in organic farming, where weed control must rely on ecological principles due to the lack of synthetic herbicides (Liebman and Gallandt, 1997). A thorough and sustainable weed management strategy that adheres to organic principles is created by combining strategic mechanical cultivation with a variety of rotations, cover crops, and intercropping (Liebman and Gallandt, 1997; Pandey S., 2024).

The "many little hammers" approach is applied in the context of organic arable farming in Skåne County, southern Sweden, through diverse rotations with cover crops and intercrops. Crop diversification influences weed communities and densities, preventing the dominance of harmful species, according to studies conducted in this area (Hofmeijer et al. 2021). Farmers seek to create an environment that suppresses problematic perennial weeds like *Sonchus arvensis* L. and lowers overall weed pressure by using intercropping techniques, competitive cover crops, and a variety of crop types. This helps to ensure the long-lasting sustainability of their farming systems (DiverIMPACTS, 2018; Uduwalage, 2024)

1.3.1 The Role of Crop Rotation Diversity

Diversified crop rotations are the basis for organic weed management. By varying the species, date of sowing, and management practices over time, farmers can disturb the life cycle of problematic weeds and prevent a single weed species from becoming dominant (Menalled et al., 2024). Well-planned rotation involves a mixture of spring and winter crops, broad-leaf and cereal species, and the strategic use of cover crops and intercrops.

There are several mechanisms by which crop rotation diversity influences weed communities:

Resource Niche Partitioning where different crops use resources (light, water, nutrients) differently and at different times, therefore reducing the resource gap exploited by weeds (Liebman and Staver, 2001).

Through phenology disruption, the different timing of tillage and sowing prevents weeds with special germination requirements from establishing a permanent population (Hald, 1999).

Biotic interactions when crops, especially legumes, may improve soil health and support beneficial microbial communities, which can inhibit weed growth (Barbieri et al., 2017).

1.3.2 Cover Crops and Intercrops as Tools for Weeds Suppression

In organic systems, cover crops and intercrops are increasingly used to provide additional weed control. Cover crops are usually grown at times when the soil would otherwise be bare, for example between main crops, to provide soil cover and to compete with emerging weeds (Teasdale, 1996). Intercropping involves the cultivation of two or more crops in the same field at the same time, which can increase resource efficiency and weed control through increased competition (Bilalis et al., 2010).

The effectiveness of cover crops in weed control is usually directly linked to the production of biomass from cover crops. High-biomass cover crops such as cereal rye and clover mixtures may produce a dense cover that effectively shades weeds and prevents them from spreading (Fernando et al., 2023). However, suppressing perennial weeds with cover crops is more difficult, as these species can often grow through the cover crop canopy and use the underground resources for survival (Melander et al., 2024).

1.3.3 The case of northern Europe

Building on the need for diversified management solutions, perennial weed management in Northern Europe presents a variety of challenges, shaped in particular by the region's unique climatic conditions. Short growing seasons, cold soil temperatures, and long, snow-blanketed winters significantly affect weed biology and limit the practical scope for non-chemical control methods (Håkansson, 2003). In this regard, species such as common couch grass (*Elymus repens* (L.) Gould subsp. repens), creeping thistle (*Cirsium arvense*) (L.) Scop., perennial sow thistle (*Sonchus arvensis* L.), and field horsetail (*Equisetum arvense* L.) are particularly problematic in Scandinavian agriculture. These weeds are widely distributed across arable land and show considerable adaptability to disturbed farming environments (Rydberg & Milberg, 2000). For example, *Elymus repens* flourishes in cereal rotations because of its large root system, which allows rapid vegetative growth and recovery after cultivation. Similarly, *Cirsium arvense* (L.) Scop. shows high persistence through its deep root system and can regenerate from small root fragments. These biological traits offer a significant competitive advantage, particularly in organic systems that do not use synthetic herbicides (Brandsæter et al., 2010).

Control strategies must therefore be based on the biological characteristics of the weed community rather than on a single general treatment.

Studies in organic systems show a trade-off between diversification, fertility building, and perennial-weed control. In Finland, a mown ley and bare fallow reduced *S. arvensis* more effectively than simply competitive crops, although bare fallow was costly (Vanhala et al., 2006). In a Scandinavian cereal experiment, red clover under sowing did not consistently suppress established *S. arvensis* or *C. arvense*, whereas post-harvest tillage had a stronger effect, particularly when shallow ploughing was followed by harrowing (Brandsæter et al., 2012). In a long-term Swedish experiment, two organic rotations showed similar overall weed pressure, while field observations and crop comparisons demonstrated the importance of field borders and crop competitiveness, with peas supporting more weed biomass than oats and winter wheat (Lundkvist et al., 2008). Rotation experiments in Denmark further showed that grain legumes could promote *C. arvense*, whereas grass-clover green manure was more compatible when it was regularly cut and mulched (Melander et al., 2016).

Furthermore, research by Tørresen et al. (2010) showed significant differences in the growth dynamics of autumn C. arvense, E. repens, and S. arvensis. While S. arvensis typically stops growing and senesces relatively early in the autumn, C. arvensis and E. repens continue to accumulate biomass for a longer period. These differences narrow the effective control window after harvest, meaning that strategies optimized for a single species may be insufficient in fields containing mixed perennial grasses, which are common in northern European agricultural landscapes (Tørresen et al., 2010).

Recent multi-site studies in Germany, Finland, and Norway have examined non-chemical strategies for controlling mixed infestations of C. arvense, E. repens, and S. arvense. These strategies included plowing, non-inverted root cutting, and competitive cover crops, used both individually and in combination. While plowing was consistently the most effective single method, integrating non-inverted tillage with competitive cover crops produced biomass reductions comparable to those achieved by plowing alone (Weigel et al., 2024).

These findings show why diversification should not be treated as a direct substitute for weed control. Its effect depends on whether the diversified rotation produces a competitive crop stand and allows timely mowing or soil disturbance.

Practices aimed at improving soil fertility, such as perennial leys or reduced tillage, could inadvertently create stable conditions that favor the prolonged growth of perennial weeds (Brandsæter et al., 2017). This leads to a fundamental compromise in organic farming in Northern Europe as methods that foster long-term soil fertility and ecosystem function can sometimes reduce weed control efficiency (Tyšer et al., 2021). Effective perennial weed management in this area therefore often requires long-term integrated strategies that extend beyond the individual growing season and are highly influenced by the specific biological and ecological characteristics of the predominant weed species, such as *Sonchus arvensis* and *Cirsium arvense (L.) Scop.*

1.4 Biology and Ecology of *Cirsium arvense* (L.) Scop. and *Sonchus arvensis* L.

This study focuses on perennial sow thistle (*Sonchus arvensis* L.) and creeping thistle, also called Canada thistle (*Cirsium arvense* (L.) Scop. because they represent some of the most economically, destructive perennial weeds for organic farming systems in southern Sweden.

Both species exhibit strong competition with crops and are able to persist through vegetative regeneration, but their root systems and seasonal growth patterns differ.

1.4.1 Sow thistle (*Sonchus arvensis* L.)

Sow thistle is a broad-leaved perennial with erect stems, milky latex, and yellow flower heads. Its persistence is mainly associated with a system of vertical and horizontal roots that bear adventitious buds (Anbari et al., 2016a). Horizontal roots can expand laterally and form clonal patches. When they are fragmented by tillage, pieces containing viable buds may produce new shoots, which means that mechanical disturbance can either weaken the population or increase the number of regenerating plants depending on the timing and intensity of the operation (Anbari et al., 2016b). The species also produces wind-dispersed seeds that may contribute to the soil seed bank and the establishment of new infestations (CABI, 2021). In spring, new shoots develop using stored root carbohydrates; later in the season, resources are redirected towards flowering and the replenishment of underground reserves (Anbari et al., 2016a).

1.4.2 Creeping thistle (*Cirsium arvense (L.) Scop.*

Creeping thistle is a dioecious perennial species, with male and female flowers occurring on separate plants. It is one of the most economically important perennial weeds because it combines vigorous above ground growth with a deep and extensive root system (Tiley, 2010). Its roots may extend more than two meters into the soil, while horizontal roots support the spread of clonal patches and provide numerous sites for shoot regeneration (Thomsen et al., 2013). Even small root fragments can produce new plants under favorable conditions (Sciegienka et al., 2011). The species is most vulnerable when its carbohydrate reserves are depleted after early shoot growth but before substantial replenishment of the root system. This stage, often called the compensatory point or compensatory stage, provides an important biological basis for timing mowing and tillage (Brandsæter et al., 2010).

1.5 Implications for the present study

The biological characteristics of these species explain why short-term weed counts alone may not provide a reliable measure of management success. A treatment may reduce the number of above ground shoots during one sampling period while leaving viable roots and buds that regenerate later. On the other hand, a treatment may increase the number of small shoots temporarily while weakening the population over time. Assessment should therefore consider weed abundance and composition together with weed biomass, crop biomass, canopy development, and crop yield.

The DRIM experiment provides an opportunity to examine these relationships in two organically managed rotation systems in southern Sweden. The Reference and Diversified rotations vary in their utilization of intercrops and diverse cover crops, allowing for the analysis of diversification at both a system and treatment level. Measurements of soil moisture, electrical conductivity, surface temperature, fractional green canopy cover, and crop chlorophyll provide non-destructive indicators of the growing environment and crop condition. When interpreted together with weed abundance, perennial-thistle dominance, biomass, and grain yield, these measurements can show whether differences between treatments are associated with crop competitiveness, canopy development, soil microclimate, or the composition of the weed community. This framework leads to the objectives and research questions presented in the following chapter.

2. STUDY OBJECTIVES AND RESEARCH QUESTIONS

This work aimed to analyze how crop diversification affects weeds at both the plot and system levels in two organically managed arable crop rotations in Southern Sweden.

To achieve this goal, the following three research questions were formulated:

Q1: How is the community composition of weed species distributed across the reference and diversified rotation systems?

Hypothesis: There will be fewer dominating weeds in the diversified system compared to the reference, but the diversified system will have a higher variety of weed species.

Q2: How do diversified treatments including intercrops and cover crops differ in weed species diversity and richness from the corresponding Reference treatments?

Hypothesis: Intercropping and adding cover crops to the rotation will increase weed species richness and diversity.

Q3: What is the relationship between weeds, particularly the perennial thistles and crop performance in the diversified and reference systems?

Hypothesis: Across the two systems, crop performance and, subsequently, yield will decrease as total weed biomass increases.

3. MATERIALS AND METHODS

3.1 Site Description

The experiment was conducted at the Alnarp SITES Lönnstorp Research Station in southern Sweden, within the Diversification through Rotation, Intercropping, and Multicropping (DRIM) Experimental Trial. The station is located at a low elevation of 7 m above sea level, at geographic coordinates 55° 39′ 21″N, 13° 03′ 30″E.

Two six-year crop rotation systems (Reference and Diversified) managed under organic certification rules were established in 2017 through collaborations among farmers, consultants, and academic experts to increase biodiversity in cropping systems by using multi-species cover crops and legumes as intercrops. The main sequence for both systems is spring mustard-winter rye-oats (undersown red clover)-red clover-winter wheat-spring pea. In the diversified rotation, blue lupin and spring barley are added as intercrops in the oats and peas, respectively. In addition, cover crop combinations of buckwheat and phacelia, and ryegrass, vetch and oil radish were incorporated into the diversified rotation (see Figures 1a and 1b).

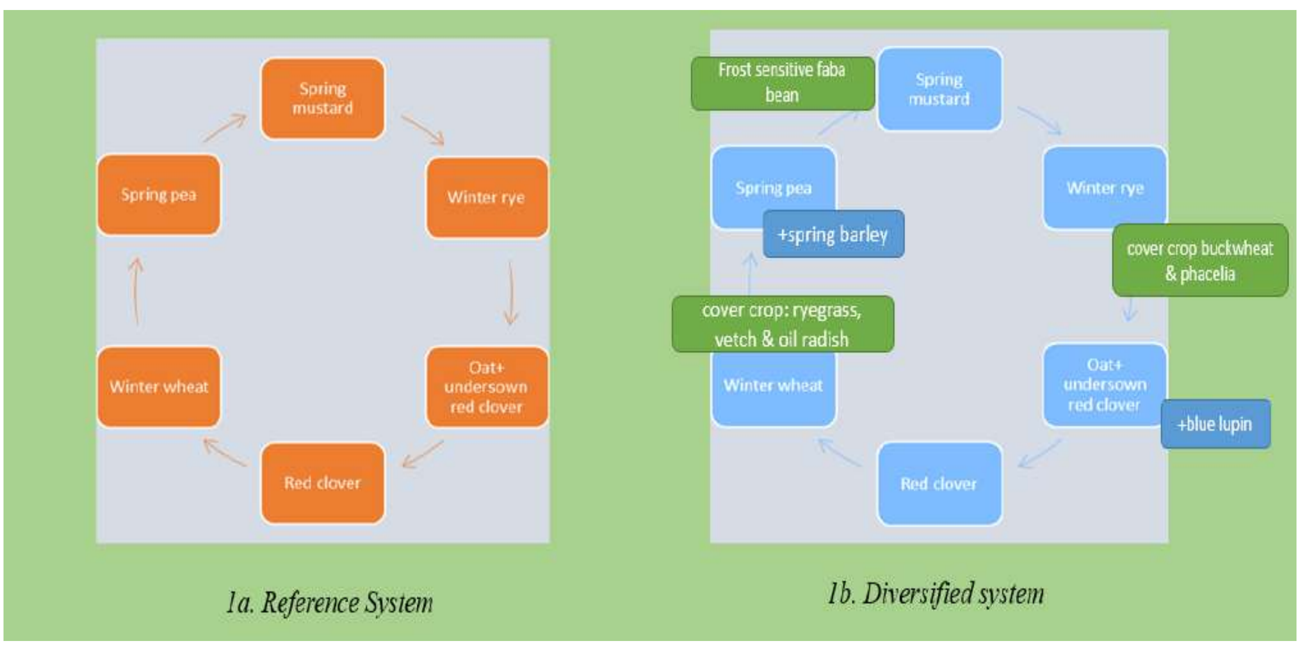

Figure 1. Two crop rotation systems used in the experiment. 1a is Reference (Ref.) and 1b is Diversified (Div.)

The Reference and Diversified treatments were designed as contrasting crop-rotation sequences rather than as a factorial experiment testing individual diversification practices. In the targeted comparisons, the preceding cover crop and subsequent intercrop were both included in the Diversified sequence, and no treatment included only one of these components. Therefore, their individual effects cannot be estimated separately. In addition, the main crop components in the intercrop treatments were planted at different densities from their corresponding sole crops, and some management practices differed between the rotation systems. Thus, all Reference-Diversified comparisons were interpreted as effects of the overall diversified treatment or rotation sequence, rather than as separate effects of the cover crop, intercrop, or plant density.

A randomized complete block design was used for this experiment. Each of the crop treatments in the reference and diversified system was replicated four times to give a total of 48 plots, each measuring 15m by 6m as shown in *Figure 2*

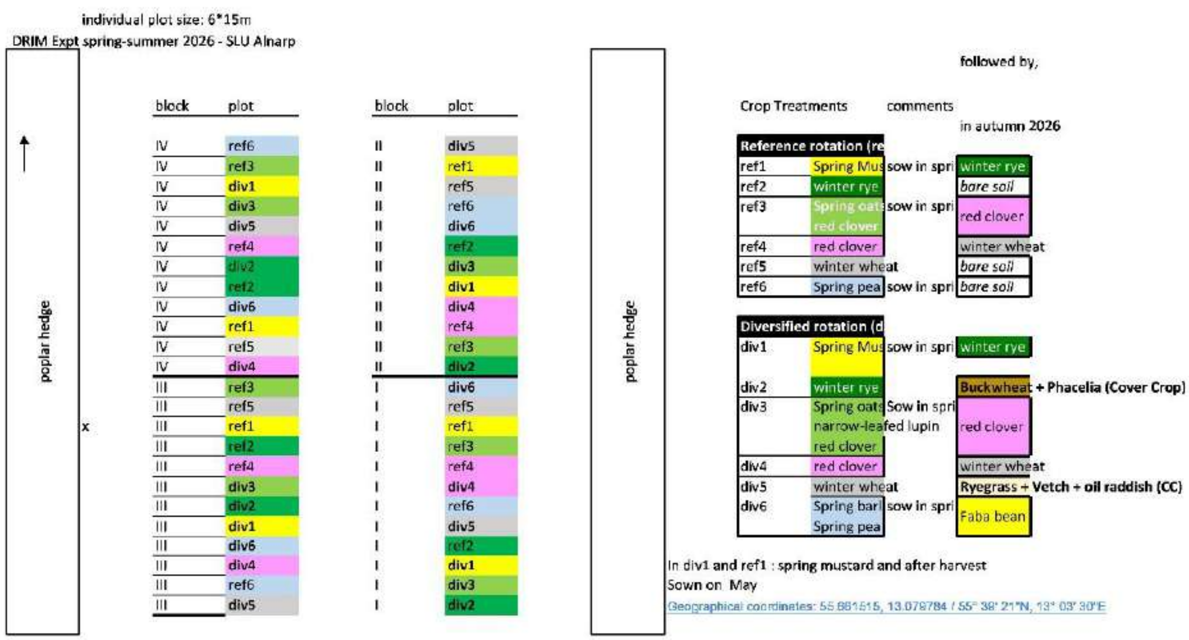

Figure 2. Field Layout showing the 48 plots arranged in a randomized complete block design in four blocks (replications). The plots are reference (ref) and diversified (div) treatments. Each colour represents a specific crop(s).

The individual 15 m × 6 m plot was the experimental unit for replicated field comparisons. Each system × crop-treatment combination occurred once in each of the four blocks, providing four plot replicates per combination where observations were available. The three marked sampling positions within a plot and the two biomass quadrats were subsamples used to characterize the plot; they were not treated as independent experimental replicates. When a variable was measured on more than one date, the observations represented repeated measurements of the same plot.

3.2 Management Practices

3.2.1 Plant densities for the sole crops and intercrop components

The establishment densities of intercrops were expressed as live plants per square meter. Reference sole pea was at 90 live plants m^{-2}, whereas pea in the Diversified pea-barley intercrop was established at 60 live plants m^{-2}, corresponding to approximately 66.7% (two-thirds) of the sole-pea rate. Barley in this intercrop was at 80 live plants m^{-2}, approximately one-third of its normal sole-crop rate. Reference sole-oat was established at 180 live plants m^{-2}, whereas oat in the Diversified oat-lupin intercrop was at 60 live plants m^{-2}, corresponding to 33.3% (one-third) of the sole-oat rate. Lupin in the intercrop was at 35 live plants m^{-2}, approximately 66.7% (two-thirds) of its normal sole-crop rate. No sole-barley or sole-lupin treatment was included; therefore, the individual performance of these intercrop components could not be compared in this experiment.

3.2.2 Fertilization

The sustainability of soils in organic cropping systems requires a strategic reduction in dependence on external fertilizers (Reimer et al., 2024; Krause et al., 2024). Bone meal fertilizer (Biofer), a slow-release organic phosphorus and nitrogen source derived from processed animal bones (Jeng et al., 2006), was applied in both cropping systems. The Biofer pellets were incorporated into the soil at sowing for all crops, ensuring direct contact with the seedbed and facilitating early nutrient uptake during crop establishment.

In the reference rotation, meat and bone meal pellets (10:3:1; N: P: K) were applied to four crops, resulting in a total nitrogen input of 270 kg N/ha per rotation. For spring mustard, 600 kg/ha of

Biofer was applied at sowing. The highest application rate, 800 kg/ha, was used for winter rye at sowing. Winter wheat and oats with undersown red clover each received 650 kg/ha, incorporated at sowing. Spring pea and red clover did not receive external fertilizer inputs due to their function as biological nitrogen fixers within the rotation (Huss-Danell et al., 2007). Relative to the reference system, the diversified rotation reduced total nitrogen input by 85 kg N/ha (31.5%), lowering it from 270 kg N/ha per rotation to 185 kg N/ha per rotation. Legume intercrops and multi-species cover crops were incorporated to enhance nutrient cycling and promote biological nitrogen fixation (Reimer et al., 2024; Rodriguez et al., 2021). The inclusion of frost-sensitive faba bean, sown in autumn prior to spring mustard, contributed additional nitrogen to the soil and reduced the required Biofer application for spring mustard from 600 kg/ha to 400 kg/ha. The amount of surface-applied bone meal for winter rye was reduced from 800 kg/ha to 600 kg/ha, based on the anticipated nitrogen contribution from faba bean residues. Intercropping blue lupin, a high-value cash crop and nitrogen fixer, with a preceding buckwheat-phacelia cover crop, decreased the bone meal application from 650 kg/ha to 200 kg/ha during the oat and undersown red clover.

Crop/ Rotation treatment	Reference Rotation (kg/ha biofer)	Diversified Rotation (kg/ha biofer)	Difference (kg/ha)
Spring mustard	600	400	200
Winter rye	800	600	200
Oat+ undersown red clover	650	200	450
Red clover	0	0	0
Winter wheat	650	650	0
Spring pea	0	0	0

Total biofer applied	2700	1850	850
Total Nitrogen (N/rotation)	270	185	85

Table 1: Biofer application rates in the Reference and Diversified System

The fertilization regime outlined in *Table 1* is based on the assumption that legume-based intercrops contribute to and facilitate the transfer of biologically fixed nitrogen within cropping systems (Massa et al. 2025; Hauggaard-Nielsen et al., 2008). Although winter wheat is preceded by a cover crop sequence of ryegrass, vetch, and oilseed radish, it still receives the full application rate of 650 kg/ha of Biofer pellets. As a primary cash crop, wheat exhibits a high nutrient demand, making yield security the principal consideration. In contrast, spring pea, intercropped with malting barley as an additional high-value component, does not require external fertilizer because the legume supplies nitrogen to both itself and its companion crop (Hauggaard-Nielsen et al. 2008; Massa et al., 2025).

Therefore, diversified crop rotation significantly enhances the sustainability of cropping systems from both agronomic and economic perspectives by shifting the nitrogen supply from costly external bone meal inputs to ecologically integrated nitrogen fixation and residue cycling (Reimer et al. 2024; Rodriguez et al., 2021). Furthermore, increased crop diversity has been shown to improve cereal productivity, resilience, and nutrient-use efficiency in organic systems (Smith et al., 2023; Krause et al., 2024). Employing biologically based nutrient management and optimizing plant-soil nitrogen interactions in organic crop rotations supports broader principles of sustainable intensification (Hefner et al., 2022).

3.2.3 Tillage

At the experimental site, a reduced-tillage strategy primarily utilizing rotor tillage was implemented at a depth of approximately 15 cm between crops, replacing the traditional plowing system. Harrowing was occasionally performed between harvest and the establishment of the subsequent crop. Additionally, during the autumn season, the soil was tilled with a cultivator, at a depth of 10-15cm. The operations were scheduled to ensure quick seedbed preparation and timely crop establishment, as harvest and sowing typically occur within a brief interval.

The introduction of cover crops and intercrops did not substantially alter the tillage system. Pea-barley intercrops received the same soil management practices as single spring pea crops and were sown at the same time. The tillage techniques applied to the cash crop were also used for the corresponding cover crops. Consequently, crop composition was primarily influenced by the diversification strategies of the rotation, rather than by changes in soil cultivation intensity or timing.

Reducing soil disturbance is increasingly recognized as an effective strategy to enhance soil quality and promote long-term system sustainability in organic farming, as demonstrated by the shift toward reduced tillage practices. Long-term research has shown that using less tillage leads to better soil structure, enhanced microbial abundance and activity, and higher levels of soil organic carbon when compared to conventional plowing methods (Krauss et al., 2020). Additionally, a meta-analysis of organic farming practices has revealed that shallow non-inversion tillage is beneficial for sustainable soil management, as it maintains crop yields and boosts soil carbon stocks (Cooper et al., 2016). Multiple studies have demonstrated that weed infestations are more prevalent under reduced tillage than under plowing, potentially leading to negative effects on crop performance and nutrient availability (Hofmeijer et al., 2019).

For thistles and other perennial weeds, plowing, which buries aboveground shoots and disrupts perennial root systems, may provide more effective control in these cases. In contrast, shallow tillage often leaves regenerative structures intact. As a result, farmers must strive to strike a balance between the environmental and soil quality benefits of reduced tillage and the potential rise in weed growth, along with the need for different ways to manage weeds. This trade-off is often the case in organic reduced-tillage approaches. Generally, the current tillage approach used can be described as a somewhat reduced-tillage system that gives priority to preserving soil while depending more on mechanical and ecological methods for weed control.

3.3 Data Collection

3.3.1 Weed Survey

Weed floras are known to differ considerably between autumn/winter-sown and spring-sown crops due to differences in disturbance timing and phenological stage (Hald, 1999; Milberg et al., 2001; Hanzlik and Gerowitt, 2016). Therefore, field scouting for weed identification and abundance was conducted in two phases, timed to coincide with the different crop sowing schedules across the 48 plots.

The first scouting was conducted on May 11 and 12, 2026, for the plots with autumn-sown crops. The second scouting took place on May 28 and 29, 2026, for the other plots with spring-sown crops. This timing allowed for the temporary covers protecting spring seedlings from rabbits to remain in place.

To capture typical weed variation in each field (Hanzlik and Gerowitt, 2016), three distinct spots in each plot were sampled. Each spot was marked with a wooden stake (*Figure 3b*) to return to the same locations for subsequent data collection. Using the same sampling points each time helps reduce spatial variability (Dodd, 2011). At each spot, weed species were identified, counted, and recorded within a 50 cm by 50 cm quadrat (Figure 3a); a standard size for these surveys (Hanzlik & Gerowitt, 2016). Sampling three points per plot helped assess variation in the weed community, including the perennial weed *Cirsium arvense (L.) Scop*. which often spreads by cloning and responds differently to changes in cropping systems.

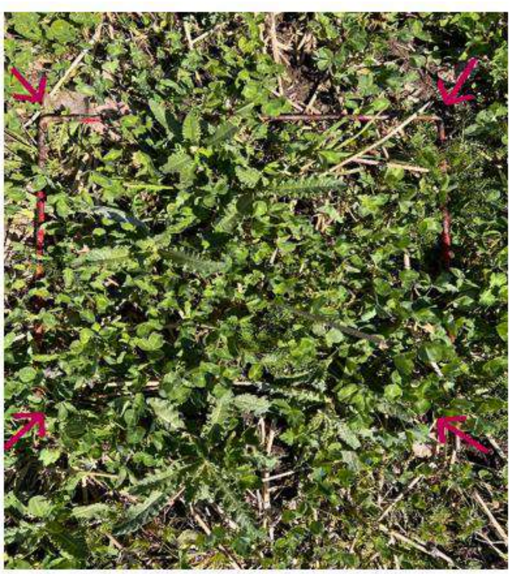

Figure 3a: 50cm x 50cm Sampling Quadrat

Figure 3b: Sampling points marked with wooden stacks

Figure 3. Weed sampling procedure

3.3.2 Field Indicators of Crop-Weed Competition

Understanding how weeds compete with crops in the field is important for research and management. In this study, we used several non-destructive indicators, including chlorophyll content, green canopy, soil water content, electrical conductivity, and surface temperature, to track crop and weed interactions. We also measured crop and weed biomass and grain yield at harvest to assess the reliability of these non-destructive methods.

3.3.2.1 Chlorophyll Content Measurement

Non-destructive optical methods for estimating chlorophyll per leaf area are common in agronomy because they closely match results from lab-based tests (Parry et al. 2014). In this study, we used an Apogee MC-100 Chlorophyll Concentration Meter (*Figure 4*) to measure chlorophyll without damaging the plants. This saved time and allowed us to monitor the same leaves over time (Masaló and Oca, 2020). We used this method on both crops and weeds. Chlorophyll data were collected from the same marked spots as the weed surveys in all 48 plots. At each spot, we measured and recorded Chlorophyll Content Index (CCI) values from both crop and weed leaves. We took several measurements from both types of plants to make the data more reliable.

Figure 4: Apogee MC-100 Chlorophyll Concentration Meter

3.3.2.2 Fractional Green Canopy Cover

Fractional green canopy cover (FGCC) is the proportion of the soil surface covered by green plants relative to the total ground area (Patrignani and Ochsner 2015). This measurement is important for tracking plant growth and light availability. To ensure spatial consistency across plot evaluations, canopy cover data were collected at pre-established marked positions within each plot, which were also designated for weed and chlorophyll sampling.

At each of the three marked sampling positions within a plot, one CANOPEO image was obtained on each of two sampling occasions. This produced 288 raw canopy-cover observations, calculated as 48 plots × three sampling points × two sampling occasions, or 144 observations per sampling occasion. The three within-plot observations were treated as subsamples and averaged to obtain one plot-level fractional green-canopy-cover value for each sampling occasion. The repeated plot-level analysis therefore used 96 plot-by-occasion values, calculated as 48 plots × two occasions.

Measurements were obtained using the CANOPEO application on a smartphone. The software calculates fractional green cover in real time by analyzing the green components of a photograph and quantifying the proportion of green pixels relative to the total pixel count (Patrignani and Ochsner 2015). To capture the images, the smartphone was placed parallel to the soil surface at a minimum height of 0.6 meters above the vegetation. Shadows were prevented from appearing in the picture to guarantee a consistent and precise view of the plot area (Patrignani and Ochsner 2015). While the app cannot differentiate between various plant types in its display, it gives a dependable, live estimation of the total green cover, indicating the general plant condition of the plot being assessed. This approach is efficient, non-destructive, and achieves precision comparable to manual, labor-intensive methods, making it suitable for frequent data collection across large experimental treatments.

3.3.2.3 Analysis of Soil Water Content, Electrical Conductivity, and Temperature

To monitor seasonal changes in soil dynamics, measurements were taken at the same marked locations within each plot, ensuring spatial correspondence with other field data. A FieldScout TDR 350 Soil Moisture Meter (Figure 5) was used to record three key variables: volumetric water content (VWC), electrical conductivity (EC), and surface temperature (Spectrum Technologies Inc., Aurora, IL, USA).

Volumetric Water Content (VWC) is the water volume per unit volume of soil and a key metric for plant-accessible moisture, influencing crop development and water-use efficiency (Robinson et al., 2008). Electrical Conductivity (EC) reflects the concentration of dissolved ions in the soil matrix and serves as a reliable indicator of salinity, nutrient availability, and overall soil health, all of which affect crop yields and weed competition (Corwin & Lesch, 2005). Soil Surface Temperature regulates essential near-surface biological processes, including seed emergence, root elongation, and microbial metabolism, making it a fundamental parameter for analyzing plant behavior (Onwuka & Mang, 2018).

Figure 5. Field Scout TDR 350 Soil Moisture Meter

Combined, these non-destructive indicators provided an overview of the soil and plant health during the study and facilitated a clearer understanding of crop and weed interactions in the two rotation systems.

3.3.2.4 Weed and Crop Biomass and Total Biomass

Crop biomass, weed biomass, and grain yield were determined from two 0.5 m × 0.5 m quadrats sampled in each plot from 20 to 23 July, except in the spring mustard and red clover treatments, which were not ready for harvest at that time. Each quadrat covered an area of 0.25 m^2, giving a total sampled area of 0.50 m^2 per plot. All plants within each quadrat were cut at ground level and separated into crop and weed fractions. Intercrop components were also separated by species from the main crop. Samples were dried at 60 °C for 72 hours, until constant mass was reached, and weighed. Cereal ears were separated from straw before threshing, whereas pea and lupin pods were shelled by hand to separate grain from the remaining above-ground biomass. Dry biomass was initially recorded in grams per 0.50 m^2 sampling area and converted to kilograms per hectare using the following equation:

$$\mathbf{kg\ ha^{-1} = grams\ per\ 0.50\ m^2 \times 20}$$

Thus, 1 g of dry mass measured over 0.50 m^2 corresponded to 20 kg ha^{-1}. Crop biomass, weed biomass, and grain yield were converted using the same area-based factor after the two quadrats had been combined to obtain one total mass per 0.50 m^2 plot sample.

Figure 6. Weed and crop biomass collection and processing

3.3.2.5 Shannon diversity index (H′)

The Shannon diversity index measures weed-species diversity in each plot by considering both the number of weed species (species richness) and how evenly individuals are distributed among those species. The index increases when there are more species and when no single species is much more common than the others. Lower values indicate only a few species or that one or a few species are much more common than the rest. The index is calculated as:

$$H' = -\Sigma (p_i \ln p_i)$$

where p_i is the proportion of individuals of species i in a plot (n_i/N), n_i is the number of individuals of species i, and N is the total number of weed individuals in the plot. In this study, we will calculate the Shannon H′ and compare it between the Reference and Diversified cropping systems to assess whether cover crops and intercrops in the diversified rotation altered overall weed-community diversity.

3.3.2.6 Pielou's evenness index (J′)

Pielou's evenness index quantifies how evenly weed individuals are distributed among species within each plot. It complements the Shannon index by separating the effects of dominance by a single species from those of species richness. A value near 1 indicates that species are present in similar numbers, whereas a value near 0 indicates that one or a few species are much more common than the rest. Pielou's evenness is calculated as:

$$J' = H' / \ln(S)$$

where H′ is the Shannon diversity index and S is the number of weed species in the plot. In this study, we will compare J′ between the Reference and Diversified systems to assess whether diversification led to a more balanced weed community, even if the total number of weeds or species remains unchanged.

3.3.3 Statistical Analysis

Data were first organized and cleaned in Microsoft Excel, then analyzed in RStudio using R version 4.3 (R Core Team, 2023). For soil variables, canopy cover and chlorophyll, each plot was treated as the experimental unit. Measurements were taken at different locations from within a plot and were averaged before analysis.

The plot-level data were analyzed using block-adjusted linear mixed-effects models that included cropping system, crop treatment, sampling period (where possible), and how they interact, while accounting for repeated and block measurements from within the plot. Estimated marginal means, Diversified-Reference comparisons, standard errors, and confidence intervals of 95% were calculated, and Holm adjustment was applied to multiple related comparisons.

Biomass and yield were available for four crop treatments while the remaining crops were not ready for harvest. Measurements from these plots were averaged across four blocks, resulting in eight aggregated system × treatment means. Comparable crops from both systems were compared directly, and intercrop treatments were assessed separately rather than together with the matching sole-crop treatment. These means were compared among the four matched crop treatments using paired t-tests, as well as exploratory regressions. As a result, biomass and yield data were interpreted cautiously.

Weed data were analyzed separately because counts had been pooled across replications and were therefore not treated as independent plot-level observations. Perennial-thistle abundance and relative abundance were compared between cropping systems using paired Wilcoxon tests across the six matched crop treatments. Species richness, Shannon diversity, and Pielou evenness were interpreted in a descriptive manner. Kruskal-Wallis tests were used only as sensitivity analyses. Correlations and regressions were used to explore relationships among chlorophyll, canopy cover, weed abundance, biomass, and yield. Because the weed data were pooled and biomass and yield were based on aggregated means, these analyses were considered exploratory. All inferential tests were two-sided, with significance assessed at $\alpha = 0.05$.

4. RESULTS

4.1 Weed Species Composition in the two systems

A total of 1,435 weeds representing 16 taxa were recorded across all sampled treatment communities. Weed abundance varied among the six crop treatments and between the corresponding Reference and Diversified communities (*Figure 7*). Reference spring mustard had the largest weed population (245 individuals), whereas Diversified Spring pea had the smallest (29 individuals). Relatively high totals were also recorded in Diversified winter rye (157 individuals) and Diversified oats + clover (149 individuals). Species composition shifted across crop treatments: *Chenopodium album* was abundant in several spring mustard, oats + clover, and winter wheat communities, whereas the combined *C. arvense* + *S. arvensis* dominated Reference winter rye, both red clover communities, Reference spring pea, and especially Diversified spring pea.

When weed counts were combined across the six crop treatments, overall weed abundance was similar between the two rotation systems: 698 individuals in the Reference rotation and 737 in the Diversified rotation (Figure 8). *Chenopodium album* represented a similar proportion of the weed flora in the Reference and Diversified systems (30.4% and 29.9%, respectively). The perennial-thistle complex was the most abundant group in the Reference rotation (213 individuals; 30.5%) and the second most abundant group in the Diversified rotation (185 individuals; 25.1%). This was the case in all six crop treatments across both rotations. The Diversified rotation had 13 recorded taxa, compared with 11 in the Reference rotation; however, these system-level totals were obtained by pooling the six treatment communities and should not be interpreted as a replicated test of species richness. The terms "crop treatment" and "treatment community" refer to the specific crop (treatment) within a rotation, whereas "rotation system" refers to the Reference or Diversified system as a whole.

The two perennial thistles did not respond consistently across the six paired crop treatments (*Figure 9*). Diversified communities had fewer thistle individuals than the corresponding Reference communities in spring mustard (5 vs. 30), winter rye (38 vs. 72), oats + clover (15 vs. 17), and spring pea (28 vs. 43). In contrast, diversified red clover had more thistle individuals (69 vs. 26), and Diversified winter wheat was slightly higher (30 vs. 25). Relative thistle dominance followed the same inconsistent pattern. In oats + clover, thistles represented a smaller share of the

Diversified weed flora than of the Reference flora (10.1% vs. 21.3%), whereas in spring pea they represented a much larger share (96.6% vs. 59.7%). The paired Wilcoxon tests detected no overall system difference in thistle abundance (V = 8.0, p = 0.600) or relative abundance (V = 9.0, p = 0.753)

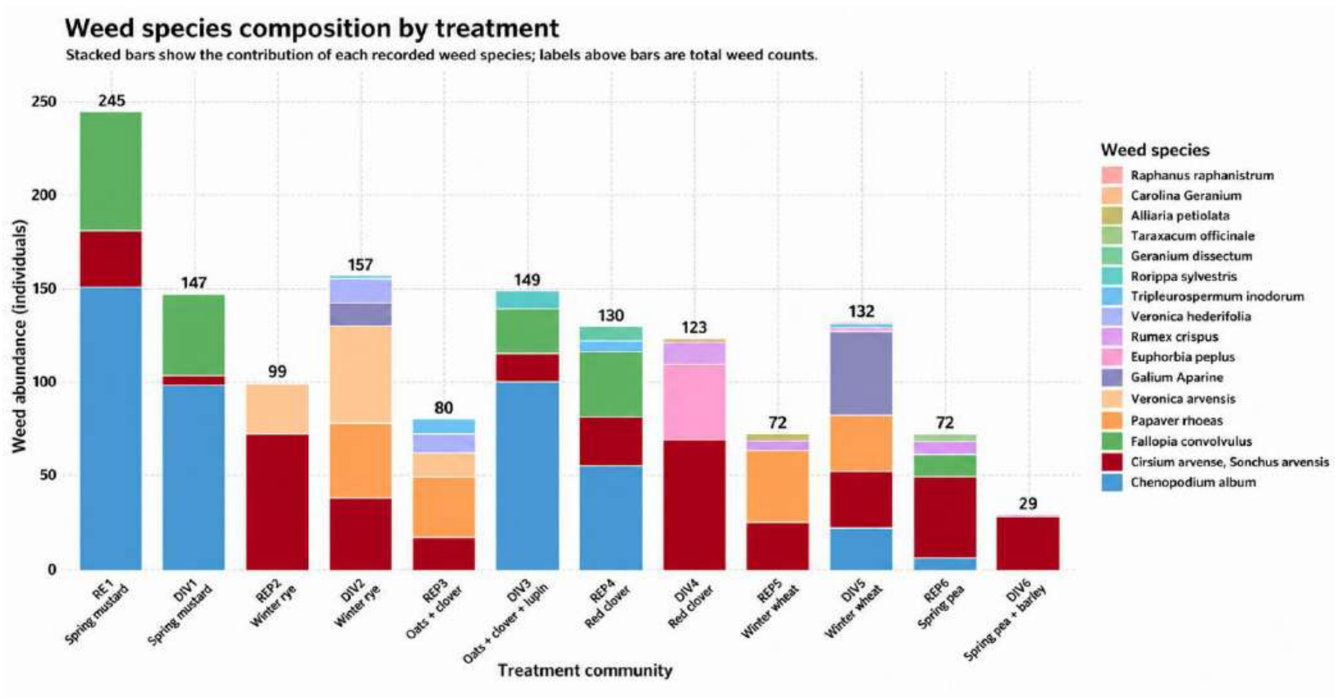

Figure 7: Weed species composition by treatment. Stacked bars illustrate weed abundance and species composition in Reference and Diversified treatment communities. Each bar displays totals from four blocks within a single treatment, with labels indicating total weed abundance. C. arvense + S arvensis is shown in red.

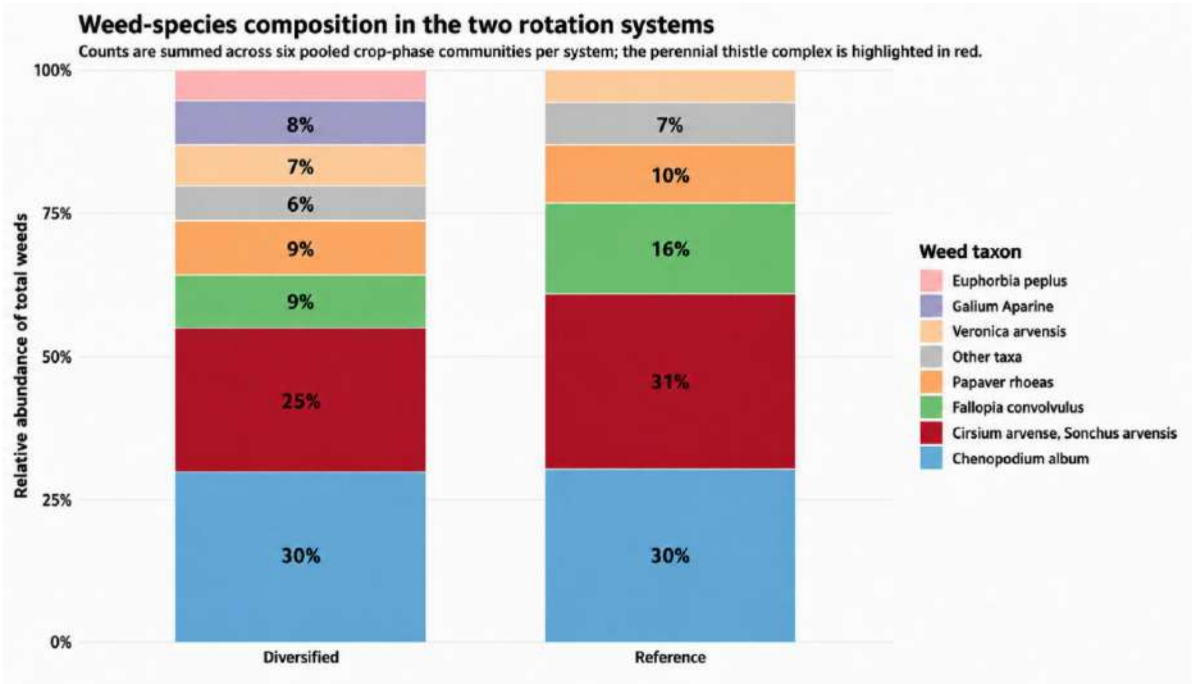

Figure 8: System-wide weed species composition in Reference and Diversified rotations. Relative abundance of weed taxa is shown using aggregated counts from six pooled treatment communities within each rotation. Percentages represent each taxon's proportion of the total weed flora, with the combined perennial-thistle complex highlighted in red.

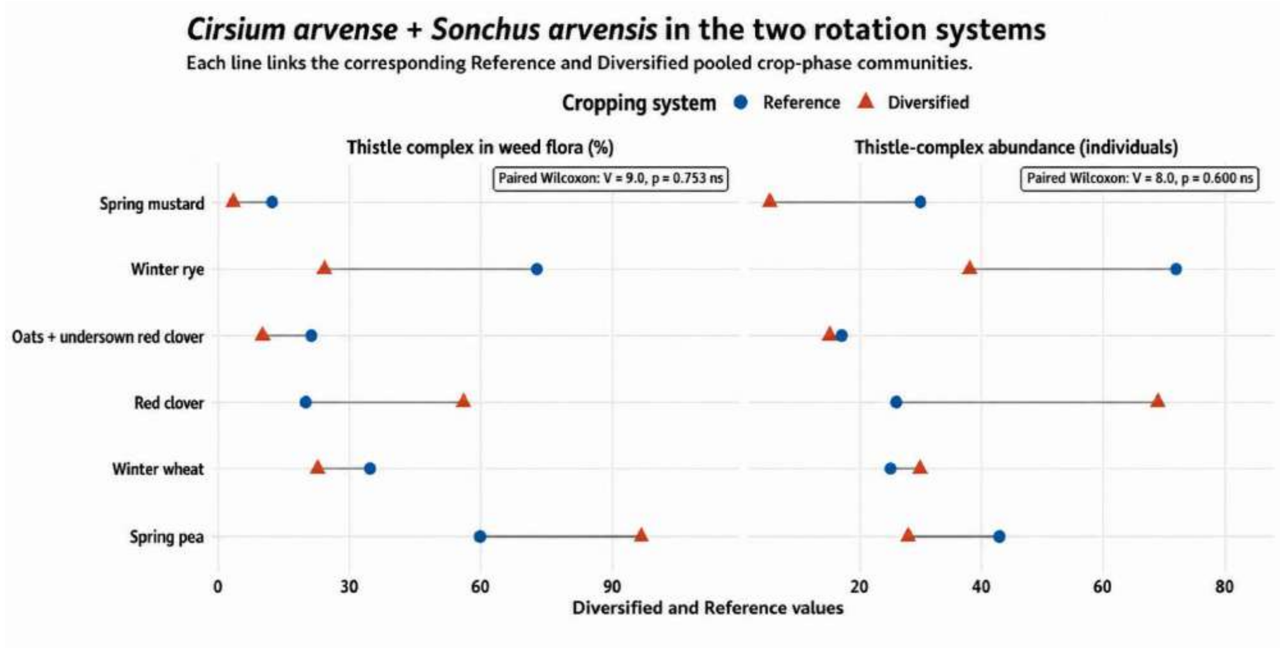

Figure 9. Perennial thistle complex across the crop rotation. Abundance and relative contribution of the combined Cirsium arvense (L.) Scop. *and Sonchus arvensis* L. *complex are depicted for six matched Reference and Diversified treatment communities. Lines connect corresponding crop treatments.*

4.2 Impact of cover crops and intercrops on weed species diversity and richness

In the oats + clover comparison (treatment 3), the Diversified community contained 4 taxa, compared with 5 taxa in Reference. Shannon diversity declined from 1.48 in Reference to 0.97 in Diversified (Δ = −0.51), and Pielou evenness declined from 0.92 to 0.70 (Δ = −0.22). The contrast was stronger in the spring-pea comparison (treatment 6): richness declined from 5 to 2 taxa, Shannon diversity from 1.20 to 0.15 (Δ = −1.05), and evenness from 0.75 to 0.22 (Δ = −0.53). Total weed abundance increased from 80 to 149 individuals in Diversified treatment 3but decreased from 72 to 29 individuals in Diversified treatment 6.

In the crop treatment 3, the Diversified treatment had slightly fewer thistle-complex individuals than Reference (15 vs. 17; Δ = −2) and a lower thistle share of the weed flora (10.1% vs. 21.3%; Δ = −11.2 percentage points). In treatment 6, the Diversified treatment also had fewer thistle individuals (28 vs. 43; Δ = −15), but the thistle share rose sharply from 59.7% in Reference to 96.6% in Diversified (Δ = +36.8 percentage points).

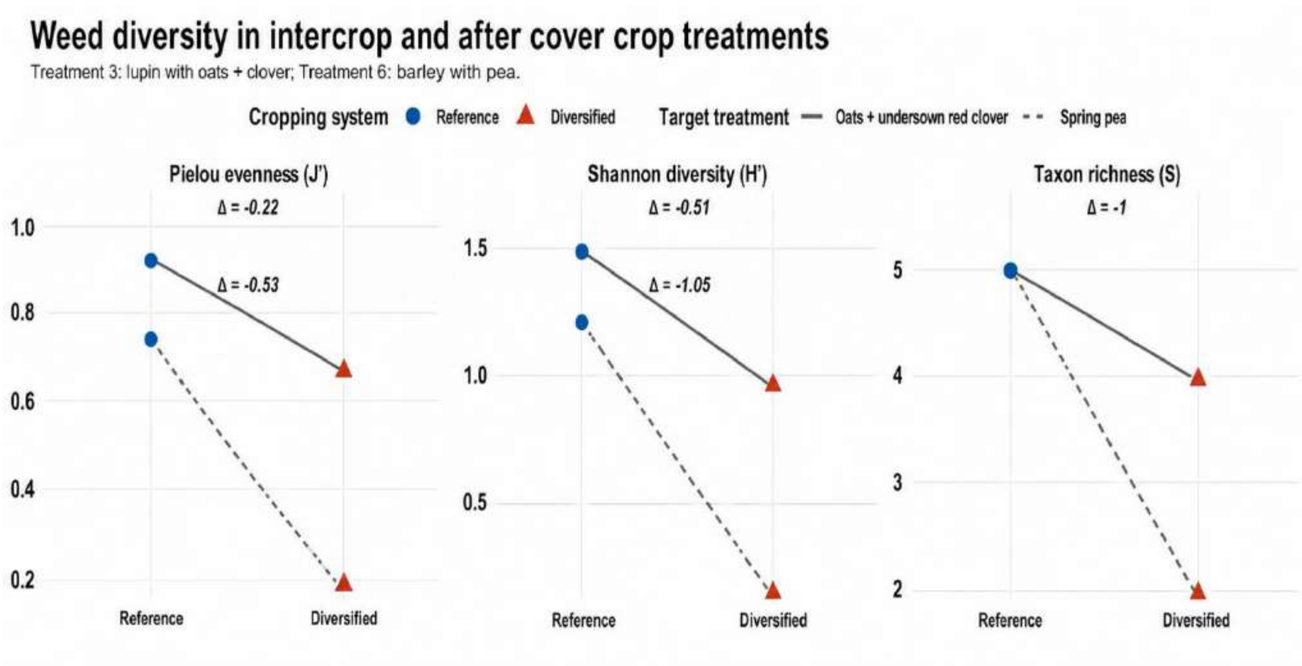

Figure 10. Weed richness, Shannon diversity, and Pielou evenness in targeted intercrop-after-cover-crop treatment, compared between Reference and Diversified oats with undersown red clover (treatment 3) and spring pea (treatment 6).

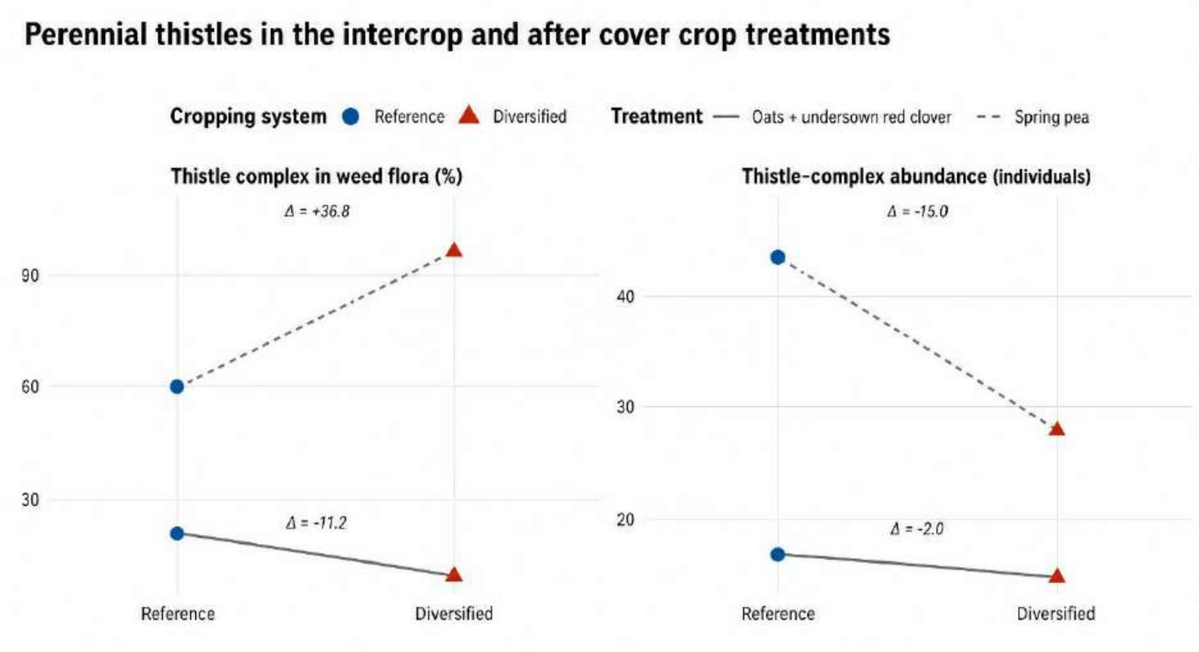

Figure 11. Perennial thistle response in targeted intercrop and after-cover-crop treatment. Abundance and relative dominance of the combined perennial-thistle complex are presented for treatment 3 and treatment 6 Reference and Diversified comparisons. Lines connect matched treatments, and Δ denotes Diversified minus Reference.

4.3 Field measurements

4.3.1 Soil moisture and microclimate dynamics across treatments and sampling phases

The reference and diversified systems had similar overall soil conditions. There was no significant difference at the system level in water content volume (VWC; $p = 0.486$), electrical conductivity (EC; $p = 0.327$), or soil surface temperature ($p = 0.177$). In *Figure 12*, all confidence intervals cross the zero line, indicating that the observed differences between systems are small and uncertain. After Holm adjustment, no comparison between crop stages was significant.

The three soil variables exhibited inconsistent behavior. VWC showed significant variation across crop stages and between the first and second sampling periods ($p<0.001$). The pattern of moisture changes over time also varied by crop stage ($p<0.001$). Surface temperature differed among crop stages and increased overall in both samples ($p<0.001$). In contrast, EC did not show any significant response to crop system, crop stage, sampling time, or their interactions (all $p > 0.15$). In summary, crop type and sampling date affected moisture and temperature, whereas EC remained relatively unaffected.

In the oats + undersown red clover, a diversified treatment included blue lupin and followed the cover crop sequence of buckwheat and phacelia. In both samples, VWC was lower in Diversified treatment 3 than in Reference treatment 3. The differences were 1.31 percentage points in the first sample and 1.30 percentage points in the second. Although the pattern was similar at both dates, neither difference was significant after Holm adjustment. Therefore, the data do not provide reliable evidence of a reduction in soil moisture due to treatment diversification.

The EC was slightly higher in Diversified treatment 3 than in Reference treatment 3, by 0.0208 mS/cm in the first sampling and 0.0250 mS/cm in the second. These differences were minor and not significant. The surface temperature in Diversified treatment 3 was 0.51° C lower in the first sample, but in the second sample the two treatments were nearly identical, with Diversified treatment 3 only 0.10° Celsius warmer. Again, neither difference was appreciable. Overall, the oats + clover + lupin treatment showed no clear differences in moisture, EC, or soil temperature compared to the reference oats + clover treatment.

In the spring pea treatment, the diversified treatment included barley and followed the sequence of rye grass, vetch and oilseed radish cover crops. In the two samples, the VWC was higher in Diversified treatment 6 than in Reference treatment 6, by 2.00 percentage points in the first sample and by 0.93 percentage points in the second. This suggests that a lower surface moisture content measured was not present in a diversified pea-barley treatment during the study period. However, the confidence intervals of both treatments exceeded zero, and neither comparison was significant, therefore the data do not report a firm conclusion that the diversified treatment improves moisture retention.

The EC values for Reference treatment 6 and Diversified treatment 6 were nearly identical in the first sample, with Diversified treatment 6 only 0.0025 mS per cm. In the second sample, EC in Diversified treatment 6 was 0.0158 mS per cm. The surface temperature was also very similar, with Diversified treatment being 0.16 °Celsius warmer in the first sample and 0.18° Celsius cooler in the second. No differences were significant. Therefore, intercropping peas and barley and the previous cover crop sequence did not result in a clear change in the three measured soil parameters compared to the reference spring pea treatment.

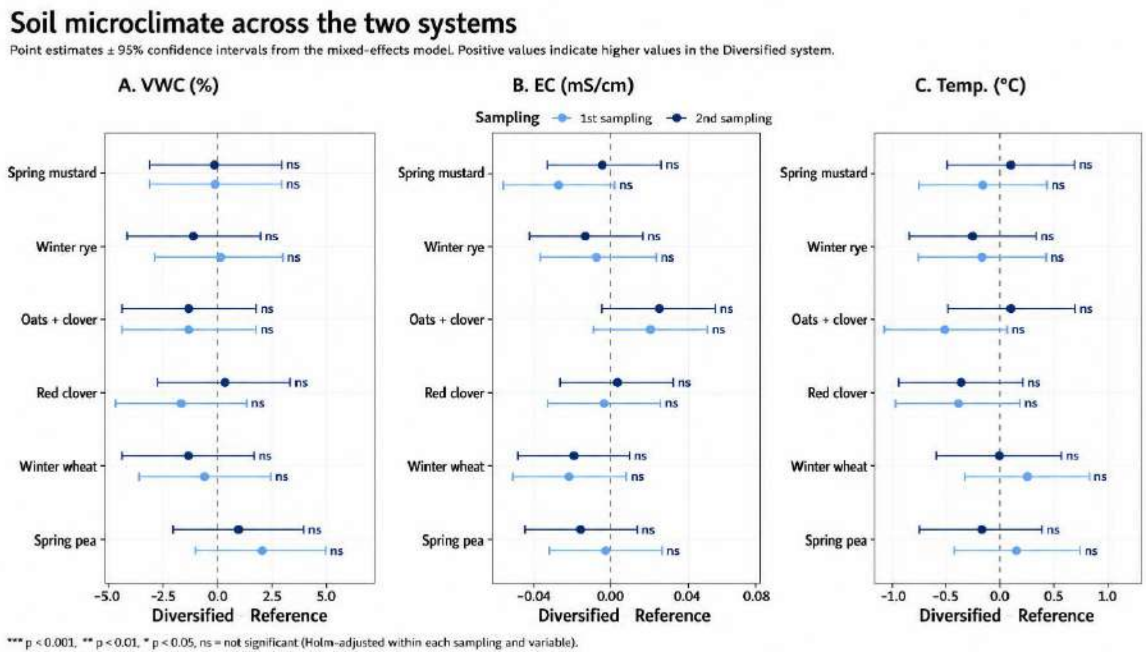

Figure 12. Estimated Diversified–Reference differences in volumetric water content (VWC), electrical conductivity (EC), and soil surface temperature across six crop treatments and two sampling occasions. Points and horizontal bars show model-estimated contrasts and 95% confidence intervals.

4.3.2 Variations in green canopy cover as a proxy for competitive canopy cover

Figure 13 shows how the CANOPEO readings varied across the two cropping systems and sampling times. In *Figure 13A*, the overall median within-plot standard deviation was 3.7 percentage points for both the Reference and Diversified systems. In several plots, the spread was less than 7 percentage points, meaning repeated measurements within the same plot were mainly consistent. However, only a few plots had more variation, with the largest spread reaching approximately 19 percentage points.

Figure *13 B* demonstrates the two green canopy-cover measurements for all the 48 plots. Thick lines mark the average for each cropping system, and thin lines show the results for each plot. At the first sampling, the average green canopy cover was nearly the same: 76.0% for the Reference system and 76.3% for the Diversified system. The 0.3 percentage-point difference was not statistically significant ($p = 0.888$). At the second sampling, canopy cover was 4.4 percentage points higher in the Diversified system, although this contrast was not significant ($p = 0.057$). The main effect of the system was non-significant ($p = 0.147$). The comparison between the two systems was equally not significant ($p = 0.147$). These results suggest that the Diversified system may maintain more green cover at the second sampling, but the evidence, however, is not strong enough to confirm an actual difference. Results for individual plots varied extensively. Some plots maintained or even increased their green cover, staying near 80–100%, while others dropped sharply, with some falling from high initial cover to below 25% at the second sampling.

Figure *13C* examines the two treatments that encompass the main diversification sequences. In the oats plus undersown red clover (Reference treatment 3) had 73.1 ± 2.7% SE green cover at the first sampling. Diversified treatment 3 (oats and clover with blue lupin after buckwheat and phacelia) had 64.7 ± 5.3% SE. Diversified treatment 3 was 8.4 percentage points lower, but this difference was not significant after Holm adjustment ($p = 0.140$). At the second sampling, both treatments increased their green cover: Reference treatment 3 reached 83.0 ± 3.3%, and Diversified treatment 3 reached 86.9 ± 2.2%. Diversified treatment 3 was then 3.9 percentage points higher, but this difference was also not significant ($p = 0.471$). In the spring-pea, Reference treatment 6 had 49.0 ± 3.6% green cover at the first sampling. Diversified treatment 6 (spring pea with barley after ryegrass, vetch and oil radish) had 51.1 ± 5.6%. The difference was small and not statistically significant ($p = 0.692$). By the second sampling, both treatments increased substantially: Reference treatment 6 reached 81.7 ± 2.6%, and Diversified treatment 6 reached 77.3 ± 2.0%. The Diversified treatment was 4.3 percentage points lower, but this difference was not significant ($p = 0.425$).

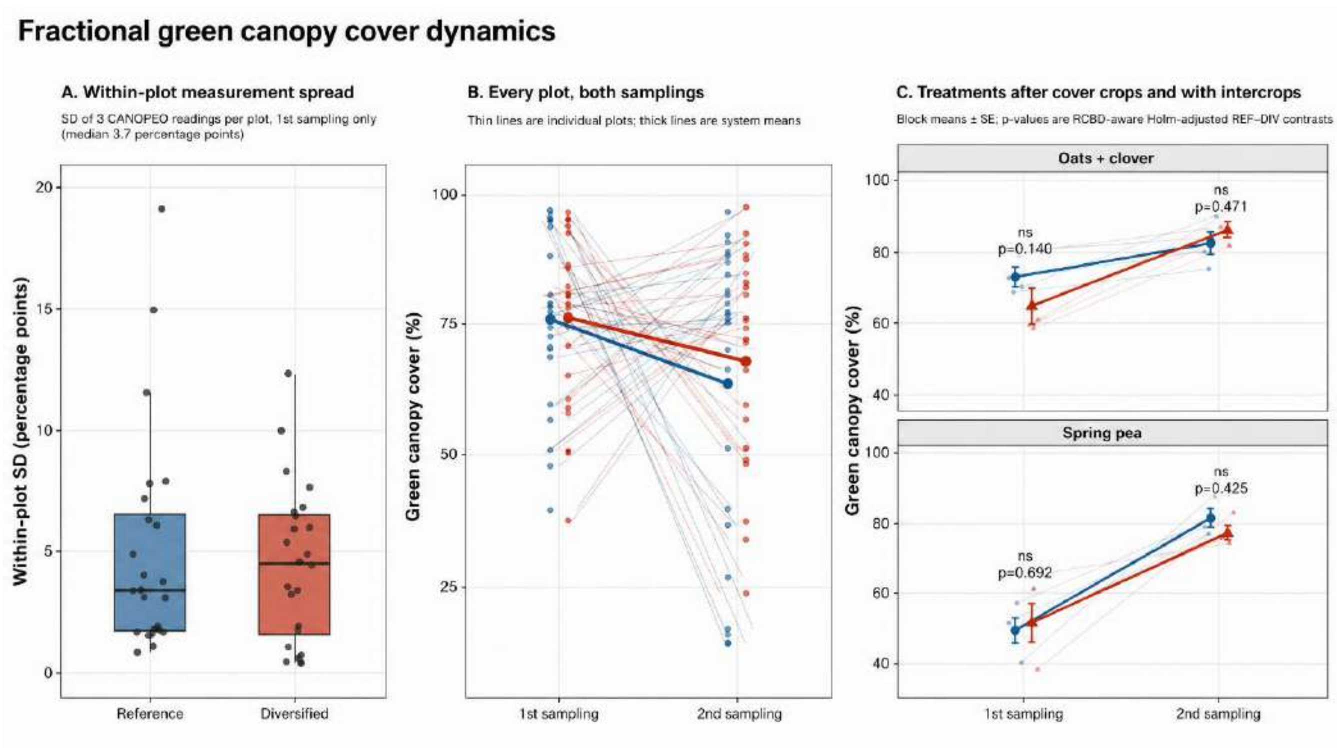

Figure 13. Fractional Green canopy-cover dynamics in Reference and Diversified cropping systems. A shows within-plot CANOPEO measurement spread, B shows all 48-plot trajectories across the two samplings, and C presents comparisons of the treatments that follow cover crops and have intercrops

4.3.3 Crop and Weed Chlorophyll Content

Figure 14 compares weed chlorophyll content (WCCI) and crop chlorophyll content (CCCI) in the Reference and Diversified systems over six crop treatments, using averages from both sampling periods. Weed chlorophyll content changed across crop treatments, with higher values in spring mustard, red clover, and winter wheat, and lower values in oats with clover and spring pea. Both systems had comparable values across treatments, and none of the comparisons between systems were significant after application of the Holm adjustment, suggesting that diversification did not steadily affect the weed community's chlorophyll status.

Crop chlorophyll content displayed clearer differences across crop treatments. Oats with clover and red clover had the highest CCCI values, whereas winter wheat and spring pea had the lowest.

These differences were associated with crop species and their growth stages at the time of measurement. Across treatments, the two cropping systems had overlapping confidence intervals and no significant differences. This indicates that crop identity had a greater impact on crop chlorophyll content than the cropping system.

In *Figure 15*, it was observed that weed and crop chlorophyll levels were largely uncorrelated across plots and sampling dates. The combined regression explained less than 1% of the variation in crop chlorophyll ($R^2 = 0.008$) and revealed a weak, non-significant correlation ($r = 0.09$, $p = 0.372$). The regression lines for both systems were nearly flat, indicating that plots with higher weed chlorophyll did not consistently exhibit increased or decreased crop chlorophyll. These exploratory regressions did not explicitly account for repeated measurements from the same plots.

This pattern remained consistent when each system was analyzed individually. The Reference system showed negligible correlation between the two indices ($R^2 = 0.002$, $p = 0.741$), whereas the Diversified system exhibited a slight positive trend ($R^2 = 0.023$, $p = 0.303$). The wide confidence intervals indicate substantial uncertainty in these estimates, especially at high weed chlorophyll levels where data points were sparse.

Figure 16 focuses on the two treatments where the Diversified system differs most clearly from the Reference system: oats + clover with blue lupin (Reference treatment 3-Diversified treatment 3) and spring pea with barley (Reference treatment 6-Diversified treatment 6), each following the relevant Diversified cover crop sequence. In the oats + clover, both crop and weed chlorophyll increased from the first to the second sampling. Diversified treatment 3 ended with slightly higher crop chlorophyll and slightly lower weed chlorophyll compared to Reference treatment 3, however, neither difference was substantial after Holm adjustment.

In the spring pea, the pea-barley treatment had statistically lower weed chlorophyll than the Reference pea at both samplings. In the two treatments crop chlorophyll increased and was slightly higher in pea + barley at the second sampling. However, these differences were statistically non-significant. Across the targeted model, crop chlorophyll increased significantly between the two samplings ($p < 0.001$), whereas WCCI showed no significant effects for system, treatment, sampling, or interaction.

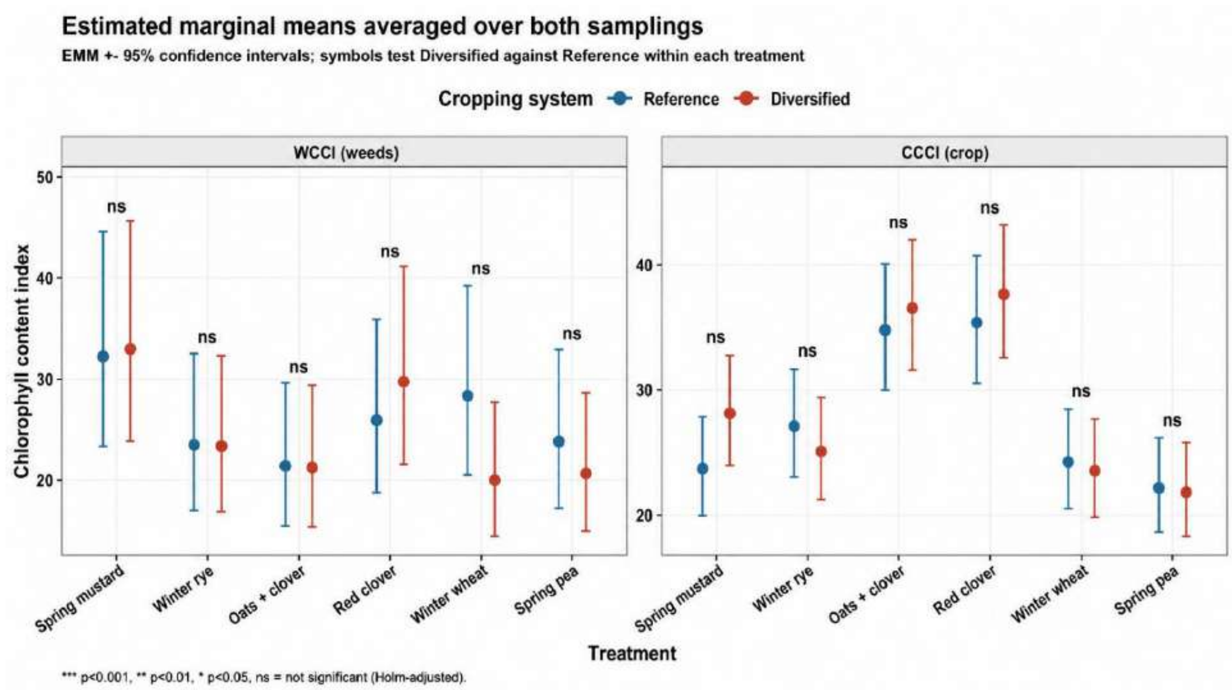

Figure 14. Estimated Marginal Means (EMM ± 95% confidence intervals) of Weeds Chlorophyll Content Index (WCCI, left) and Crop Chlorophyll Content Index (CCCI, right) across six crop treatments in Diversified (red) and Reference (blue) cropping systems

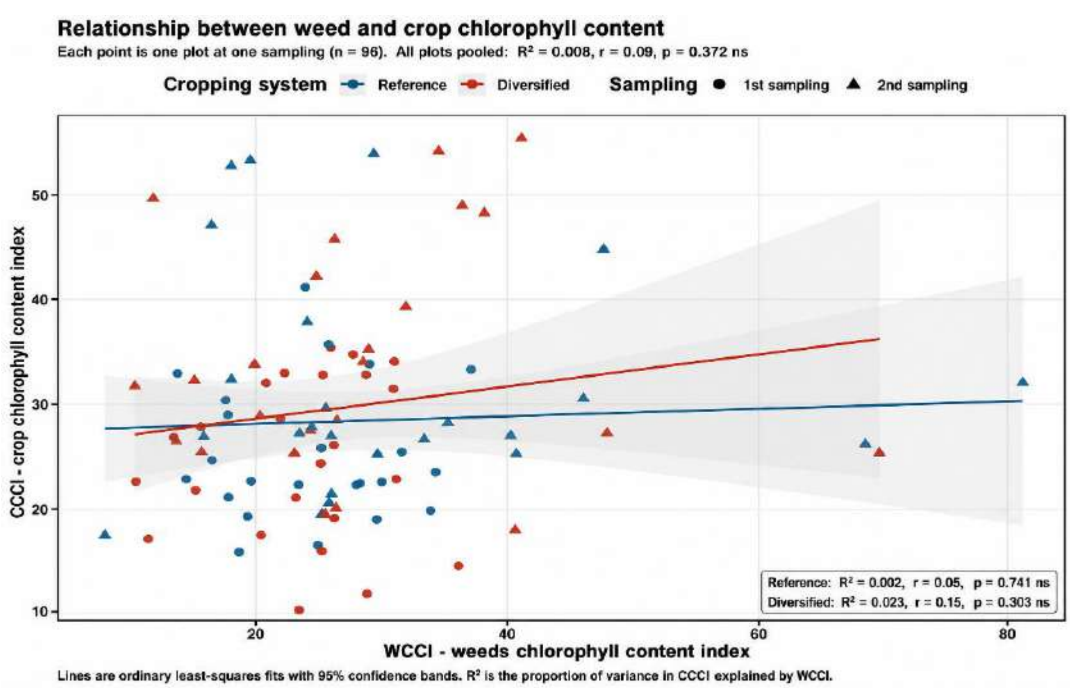

Figure 15. Scatter plot showing the relationship between the Weeds Chlorophyll Content Index (WCCI) and the Crop Chlorophyll Content Index (CCCI) across all plots for 2 sampling periods. Solid lines show linear trends for each system, with 95% confidence intervals (shaded bands).

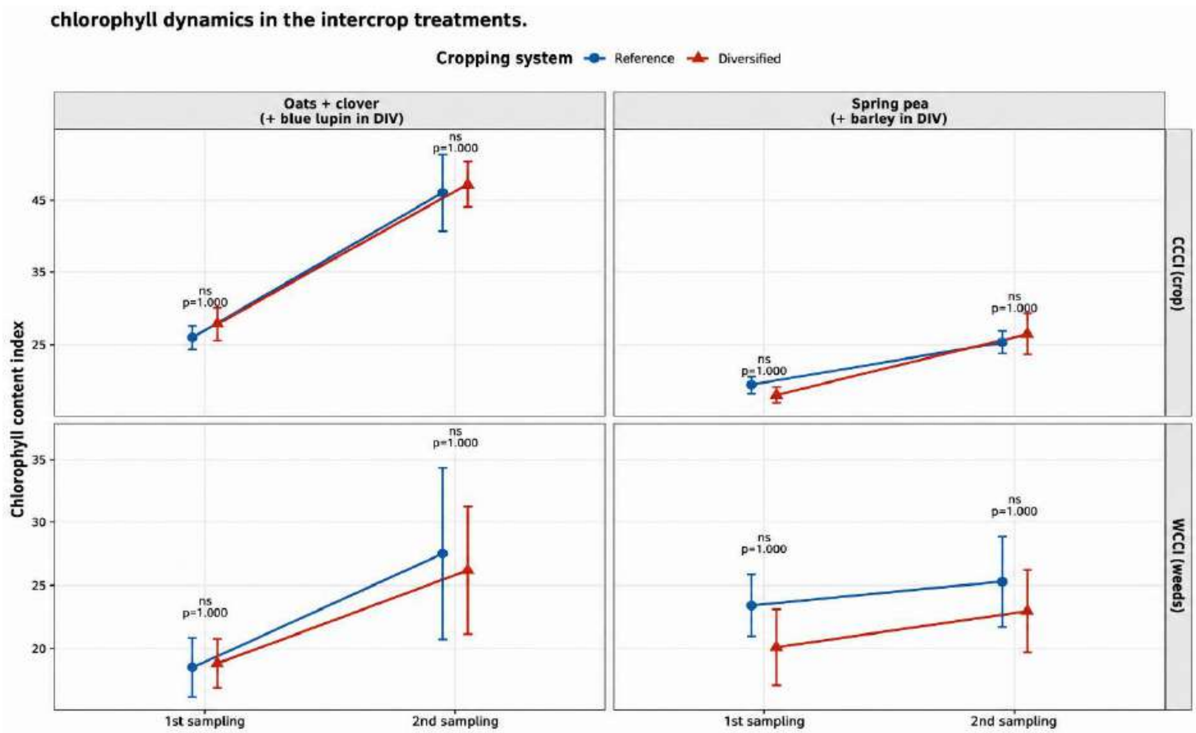

Figure 16. Weeds (WCCI) and crop (CCCI) chlorophyll dynamics during the diversified intercrop-after-cover-crop treatments. Points and error bars show the mean ± SE across four RCBD blocks for REF3 versus DIV3 (oats + clover with blue lupin in DIV) and REF6 versus DIV6 (spring pea with barley in DIV)

4.3.4 Crop performance and weed suppression dynamics

Crop biomass, weed biomass, and grain yield varied more among crop treatments than between the two cropping systems overall (*Figure 17*). The system comparisons shown here are based on matched Reference and Diversified treatment summaries across the four harvested crop treatments. The individual plot was the experimental unit in the field design, whereas the values displayed for each system × treatment combination are summaries across the four blocks. The exploratory paired comparisons across the four aggregated treatment pairs did not indicate significant overall differences in crop biomass ($p = 0.985$), weed biomass ($p = 0.603$), or grain yield ($p = 0.903$).

In crop treatment 2, winter rye performance was similar across systems. Diversified rye had 7,260 kg/ha of crop biomass and 3,658 kg/ha of grain yield, compared with 6,856 kg/ha and 3,550 kg/ha in Reference rye; weed biomass was also similar, at 2,177 and 2,274 kg/ha, respectively. In crop treatment 5, winter wheat also showed similar crop biomass and grain yield in both systems, although weed biomass was lower in Diversified wheat (2,385 kg/ha) than in Reference wheat (2,872 kg/ha).

In crop treatment 3, the oat component of the Diversified oat-lupin treatment produced 7,742 kg/ha of biomass and 4,379 kg/ha of grain, compared with 11,664 kg/ha and 6,580 kg/ha, respectively, for sole-crop oat in the Reference treatment. Thus, at the lower oat seeding density used in Diversified treatment 3, oat produced 66% of the Reference oat biomass and 67% of the Reference oat grain yield. Lupin had an additional 608 kg/ha of crop biomass and 213kg/ha grain yield to the oat, but no sole-lupin treatment was available, so its performance cannot be compared. Weed biomass was higher in Diversified treatment 3 (945 kg/ha) than in Reference treatment 3 (240 kg/ha). For the peas in diversified treatment 6, 2273 kg/ha crop biomass and 905 kg/ha grain was produced compared with 4,068 kg/ha and 809 kg/ha in Reference treatment 6, despite weed biomass being lower in Diversified treatment 6 (816 kg/ha) than in Reference treatment 6 (1,740 kg/ha). The barley intercrop contributed 4,771 kg/ha of crop biomass and 2,452 kg/ha of grain yield to the Diversified treatment total; it was not compared directly with pea because the crops were different species.

Figure 18 shows eight aggregated system × treatment means, comprising the Reference and Diversified summaries for four harvested crop treatments. Each value was aggregated across the four blocks; therefore, these eight values are not individual plot replicates. At this aggregated treatment level, crop biomass was negatively associated with weed biomass ($R^2 = 0.579$, $p = 0.028$) and positively associated with grain yield ($R^2 = 0.969$, $p < 0.001$). These ordinary least-squares regressions are exploratory and describe relationships among treatment means. They do not establish cause and effect, and their strength is limited because block-level variation is not represented in the eight-plotted observations.

In *Figure 19*, we see that moving from Reference treatment 3 to Diversified treatment 3 resulted in a reduction of 3,314 kg/ha in total crop biomass and 1,988 kg/ha in grain yield, accompanied by an increase of 706 kg/ha in weed biomass. These results indicate that the oat-lupin mixture produced lower crop yields and was less effective at suppressing weeds compared to sole oat.

In crop treatment 6, the results contrasted with those observed in crop treatment 3. Diversified treatment 6 exhibited 2,975 kg/ha greater total crop biomass and 2,548 kg/ha higher grain yield than Reference treatment 6, while weed biomass was 924 kg/ha lower.

Figure 20 shows the establishment-density context for the component comparisons in *Figures 17* and *19*. A sole-crop oat at 180 live plants/m² was planted as a Reference treatment 3. Diversified treatment 3 included 60 oat plants/m² and 35 lupin plants/m²; therefore, oat was recognized at one-third density of the Reference oat. Lupin was an intercrop hence had no comparison.

Another sole-crop, pea, was planted at 90 live plants/m² as Reference treatment 6. Diversified treatment 6 included 60 pea plants/m² and 80 barley plants/m². Therefore, Pea was established with a density of about two-thirds of the Reference pea. The pea-barley treatment produced greater total crop biomass and grain yield and lower weed biomass than sole pea, but barley yield was not compared directly with pea yield because the crops are different.

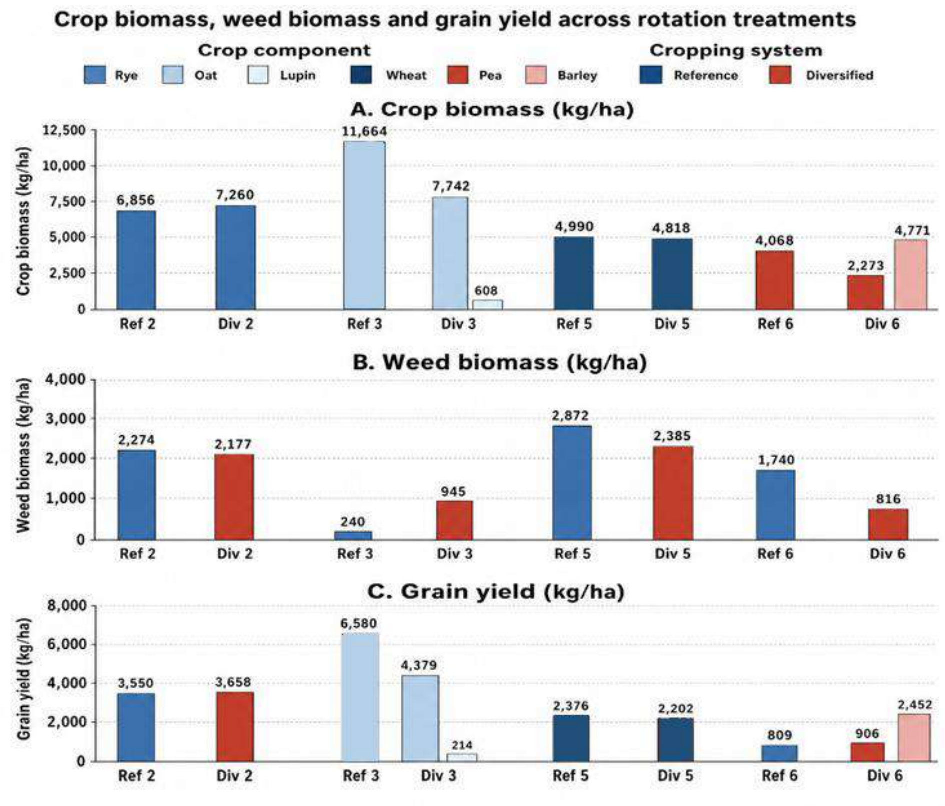

Figure 17. Crop biomass, weed biomass, and grain yield across rotation phases in the Reference and Diversified cropping systems. ***.*** *Diversified intercrop components are shown separately for oats + clover with lupin (Div 3) and spring pea with barley (Div 6)*

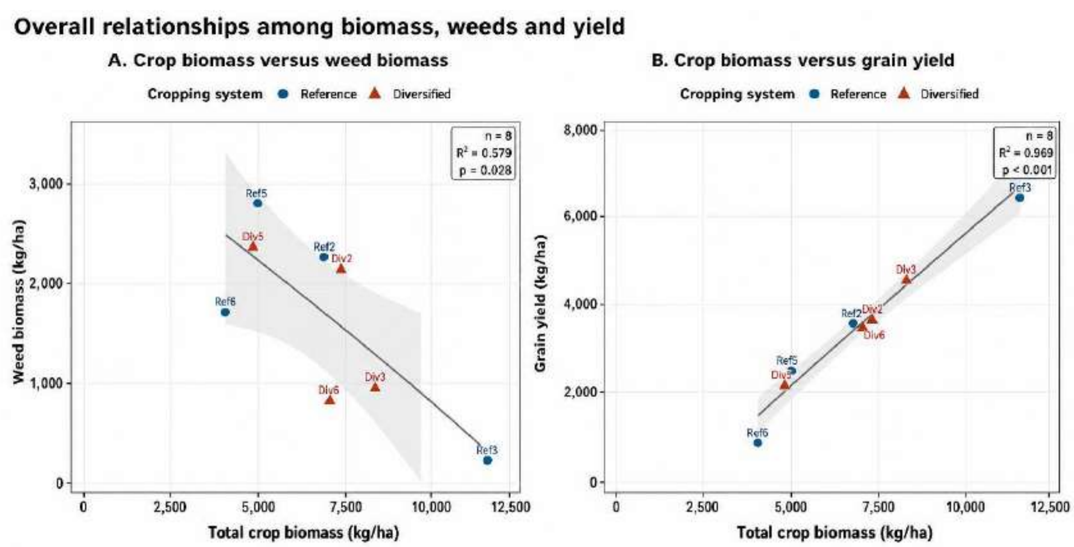

Figure 18. Exploratory relationships among crop biomass, weed biomass, and grain yield across eight aggregated system × crop-treatment means. Each point represents one Reference or

Diversified treatment value accumulated across four randomized complete blocks for one of the four harvested crop treatments (n = 8 aggregated values). Lines show descriptive ordinary least squares regressions of 95% confidence intervals. The regressions summarize relations among treatment means. However, these are not confirmatory tests of causal treatment effects.

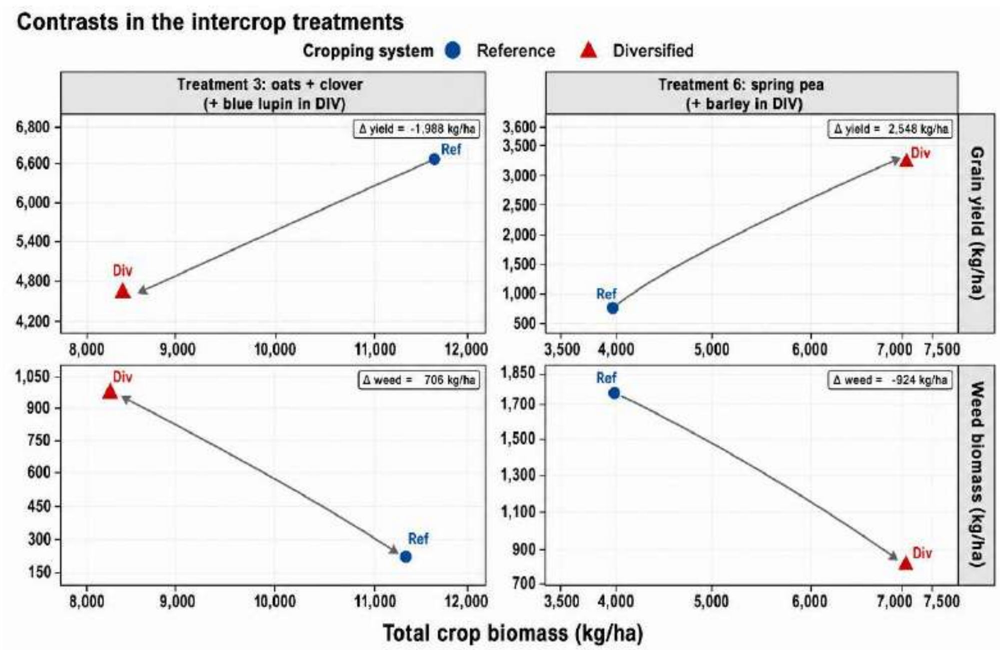

Figure 19. Biomass trends in the diversified intercrop treatments. Lines connect the corresponding Reference and Diversified whole-plot totals for oats + clover with blue lupin (Treatment 3) and spring pea with barley (Treatment 6).

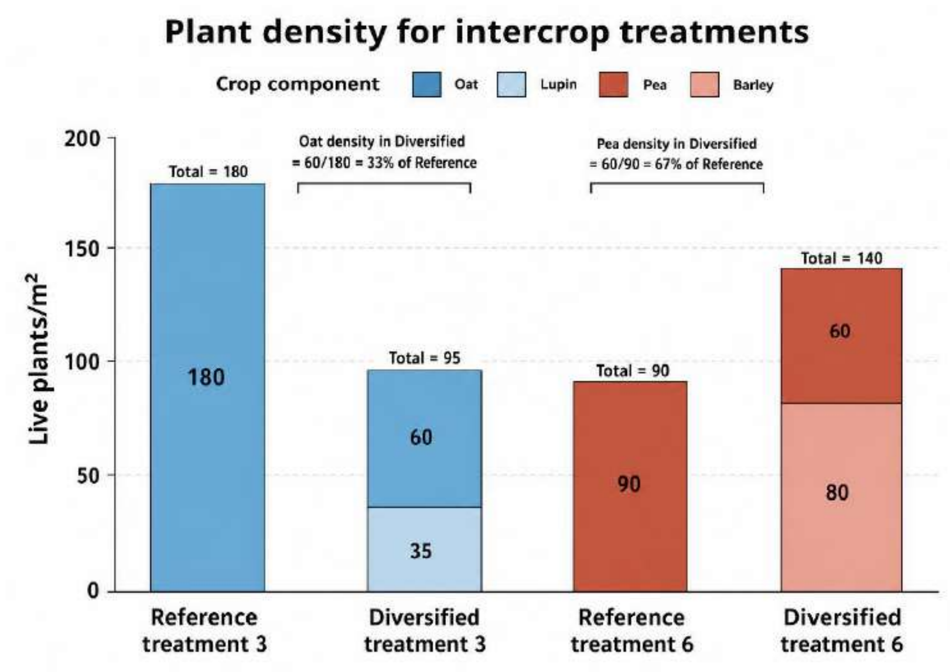

Figure 20. Initial plant establishment in the intercrop treatments showing the live-plant density of sole-crop and intercrop components

5. DISCUSSION

5.1 Weed species composition across the Reference and Diversified systems

The finding that the composition of weed communities was influenced more strongly by crop treatment than by the rotation system indicates that the crop and management context mattered more than, whether it was simply a Reference or Diversified system. This finding is in line with the literature which shows crop type, sowing time, canopy development, and management sequence. And this can shape weed communities more strongly than species richness in the rotation by itself (Smith & Gross, 2007; Hofmeijer et al., 2021; Nguyen & Liebman, 2022). The two systems therefore shared several taxa, but individual crop treatments created different openings for annual weeds or perennial thistles to become dominant. This can be interpreted as evidence that diversification changes weed pressure through the conditions created by particular crop combinations, rather than through a uniform system-wide effect.

At the system level, diversification did not produce a consistent reduction in weed dominance or a statistically supported increase in weed diversity. The initial hypothesis that the Diversified rotation would contain fewer dominant weeds and a broader weed flora was therefore not supported. This result should not be interpreted as proof that diversification is of no importance. Weed communities respond to preceding crops, soil disturbance, seed-bank dynamics, and the survival of established underground structures, and these processes may require several rotation cycles before system-level differences become detectable. The absence of a short-term system effect is therefore compatible with evidence that the outcome of rotation diversification depends on its timing, crop competitiveness, and implementation rather than on the number of crop species alone (Weisberger et al., 2019; Hofmeijer et al., 2021).

For *C. arvense*, no single annual practice is expected to offer consistent long-term control; instead, a combination of competitive crops, cover crops, mechanical methods, and repeated stress on root reserves is necessary (Favrelière et al., 2020). This explains why perennial thistles remained robust in both rotations. Farmers are not only concerned about the variety of weed species in their fields but also about the costs and challenges associated with controlling the ones that persist. Perennial thistles can survive through vegetative propagation and may reappear after a crop cycle that temporarily diminishes their above-ground presence. Therefore, a short-term reduction in the total number of weeds should not be mistaken for successful weed management, especially where *C. arvense* or S. arvensis dominate the remaining weed community. The results from weed species composition demonstrate that the Diversified rotation did not eliminate the thistle problem; rather, it changed the conditions under which thistles and annual weeds became dominant.

5.2 Intercrops, cover crops, and weed diversity

The comparisons of intercrop treatments showed that diversification did not automatically increase weed richness or diversity. In both crop treatment 3 and crop treatment 6, the Diversified communities had lower diversity than the Reference, therefore, the second hypothesis was not confirmed. However, the ecological meaning of lower diversity differed between the two treatments: in crop treatment 3 it reflected stronger dominance by an annual weed, whereas in crop treatment 6 it reflected stronger dominance by the perennial thistles. A lower Shannon index is therefore not sufficient evidence of successful weed management. The dominant taxa and its persistence must also be considered. The results are consistent with literature emphasizing that weed-community outcomes depend on crop sequence, mixture design, and management timing rather than on diversity per se (Gu et al., 2021; Nguyen & Liebman, 2022).

The preceding cover crops could contribute to these results through the changes in residue cover, nutrient cycling, soil disturbance, and establishment environment, but it is worth noting that this design does not allow separation of individual effects of the preceding cover crop and intercrop. The observed differences reflect the effect of the entire Diversified sequence, involving both the preceding cover crop and the following intercrop. Long-term comparison trials are needed for identifying the contribution of each practice.

5.3 Contribution of the diversified species

The species in the Diversified rotation perform different roles. This results in the possibility of having a combination of rapid ground cover, nutrient capture, biological nitrogen fixation, and complementarity in the mixture, but it also implies that the success of the combination depends on its ability to maintain a competitive canopy at the moment of weed establishment.

In the case of the oat-lupin mixture, we see why it is necessary to consider both the functional role of a plant species and its seeding rate. For instance, although the lupin is a nitrogen-fixing legume, it cannot be expected to form an early canopy closure and perform direct nitrogen fixation in the same way as the oat. In the lupin-cereal intercrop, cereals play the leading role in providing competition with weeds at the early growth stages, and Carton et al. (2020) report that the fast growth rate of cereals and rapid soil-nitrogen uptake compensated for the lower weed-suppressive qualities of lupin in the mixture with triticale.

Diversified treatment 6 mixture represents the complementary case: pea provides a legume component capable of nitrogen fixation while barley provides a rapid early ground cover, high soil-nitrogen acquisition, and competitive cereal canopy. Higher biomass of crops, lower biomass of weeds, and higher yield of total grain in the intercrop are in accordance with such interpretation that allows a more complete canopy occupation. This is supported by a meta-analysis by Gu et al., (2021) which demonstrated that weed-suppressive qualities of the intercrops depend on the mixture design and the competitiveness of the component species, not diversity by itself.

The cover crops need to be understood as preparations rather than sure means of weed control. The weed suppression capacity of buckwheat relies on its quick soil cover capacity (Björkman, 2013), and phacelia has revealed species-specific weed suppression and nutrient cycling roles in cover crop comparison (Cottney et al., 2020). Oil radish can suppress weeds in the early stages by growing quickly rather than having constant allelopathic effects (Lawley et al., 2012). The role of ryegrass will be that of a non-legume nutrient scavenger and soil cover element, while vetch fodder will provide a nitrogen-fixing legume role. Therefore, the combination of ryegrass, vetch, and oil radish will enable the retention of nutrients in the form of biomass, as well as soil cover, although living cover utilizes water resources. That is why a cover crop can reduce soil evaporation post-termination without increasing the soil water content for the next crop at all measured depths and sampling dates (Wang et al., 2021; Garba et al., 2022).

5.4 Soil moisture and microclimate

The results revealed no general differences between the Reference and Diversified systems in terms of soil volumetric water content, electrical conductivity, or surface temperature. In most cases, the differences were small, and the confidence intervals crossed zero. The intercrop comparisons were also non-significant. In other words, even if cover crops and intercrops can influence ground cover and soil-surface conditions, the additional farming practices did not generate a consistent difference in the soil microclimate during any of the two spring sampling times.

It is necessary to consider this finding with regard to the impact of cover crops on the water balance. Cover crops can use water via transpiration, while the cover of cover crops and their residue will shade the surface, decrease evaporation, increase infiltration, and increase soil water storage near the surface. The net effect depends on the weather, soil texture, cover crop species, amount of biomass, timing of termination, and residue management. According to Wang et al. (2021), cover crops could lead to decreased evapotranspiration and increased soil water storage near the surface while decreasing the water storage within the whole profile or decreasing precipitation-storage efficiency due to water use by the living cover crop. Garba et al. (2022) also note that the impact of cover crops on the soil water content and yields is context-dependent, especially for water-limited environments.

The lack of differences in soil properties in our research thus does not conflict with the known mechanisms of action of cover crops. It means that the mechanisms did not work to a noticeable degree on the sampling dates or were counterbalanced by water use and crop-phase differences and that the crop and weed impacts should be explained by the impacts of density, crop composition, canopy coverage, and weed communities rather than a single soil microclimate factor.

5.5 Green canopy cover as an integrated proxy for crop competition

In relation to crop-weed competition, the results make a valuable connection between soil and canopy dynamic. The two systems started with similar average of fractional green canopy coverage at the first sampling. By the second sampling, the Diversified system kept slightly higher green cover at the field level; however, the difference was not statistically significant. Individual trajectories were highly diverse, since six crop stages differed greatly in development and had different dynamics in green cover growth because of crops' growth or senescence. There were no differences in canopy cover in the intercrop treatments.

CANOPEO determines fractional green canopy cover by colour thresholds in images (Patrignani & Ochsner, 2015). Thus, it performs similarly to NDVI and other greenness indices: CANOPEO provides quick and integrative estimate of the proportion of ground covered with green vegetation and may be used as a proxy for canopy growth and light interception potential. However, CANOPEO does not differentiate crop green tissue from weed green tissue. Thus, a high canopy cover can represent strong crop canopy cover, high weed cover or a combination of both. It should not be taken as evidence of crop competitiveness without the corresponding information on weed biomass, crop biomass and species composition.

Despite the lack of distinction between crops and weeds, when considered together with other figures, canopy cover remains quite informative. Treatment-level exploratory analysis demonstrated a strong negative correlation between perennial thistle relative abundance and mean green canopy cover (Spearman rho = −0.80; FDR- adjusted $p = 0.046$; $n = 12$). This is consistent with the assumption that the weak canopy cover leaves room for thistles to dominate. However, we cannot determine cause and effect from this data. High crop canopy may prevent thistle's appearance, but thistle dominated communities may also be a result of poor crop cover. Evers and Bastiaans (2016) proved that crop density and canopy structure are important in crop-weed competition, supporting the use of green cover as a substitute rather than a stand-alone weed-control measure.

5.6 Chlorophyll indices

The correlation coefficient for WCCI and CCCI across the whole study (pooled $R^2 = 0.008$, $p = 0.372$) was negligible. Consequently, the results failed to confirm the interpretation that the Diversified system changed crop and weed physiology during the sampling period. These findings should not be perceived as uninformative. While the chlorophyll meters provide a fast and non-destructive measure of leaf chlorophyll state, the relation of that measurement to the actual concentration of chlorophyll in leaves depends on the species and optical characteristics of the leaves (Uddling et al., 2007).

However, the leaf greenness does not equal biomass, canopy occupation, resource capture, or grain yield. High chlorophyll content does not necessarily mean that the plant occupies the sufficient amount of space to suppress weeds. On the contrary, a stand can suppress weeds by early light interception without a visible reaction of chlorophyll content.

This explains why the chlorophyll data complement and do not contradict the biomass data. The negligible WCCI-CCCI association indicates that the competitive process was not detected by these leaf-level chlorophyll indices during the sampling period.

The ecological signal appeared at the stand level, as higher crop biomass was associated with lower weed biomass and higher grain yield. In our experiment, chlorophyll turned out to be useful as it showed that the two systems did not differ systematically in terms of short-term leaf greenness.

5.7 Crop performance, weed suppression, and grain yield

Under the establishment densities used in this experiment, the Diversified oat treatment produced lower total crop biomass and grain yield, but higher weed biomass, than the Reference sole-oat treatment. In contrast, the pea component produced lower biomass than sole pea, whereas the complete pea–barley stand produced greater total crop biomass and grain yield and lower weed biomass than the Reference sole-pea treatment. These exploratory comparisons represent complete treatment sequences rather than isolated tests of intercrops, or seeding-density effects. Crop identity, component density, preceding cover crop, and associated management differed simultaneously between treatments; therefore, the observed differences cannot be attributed to any single component or factor.

6. CONCLUSION

In summary, crop diversification did not consistently change weed composition, dominance, richness, or diversity at the system level; instead, weed communities responded mainly to individual crop treatments. The first two hypotheses were therefore not supported, and the persistence of perennial thistles showed that greater crop diversity alone was insufficient for weed control.

The clearest relationship was between crop performance and weed pressure. Greater crop biomass was associated with lower weed biomass and higher grain yield, supporting the third hypothesis for total weed biomass, although the relationship with perennial thistles remained less certain. The contrasting intercrop responses indicate that planting density, crop compatibility, and canopy competitiveness mattered more than species number, consistent with evidence that intercrop weed suppression depends on mixture design and density (Gu et al., 2021). The lack of consistent soil-microclimate and chlorophyll responses further suggests that the main effect occurred at the crop-stand level.

These findings are descriptive because planting density, crop identity, intercrop composition, and preceding cover crops could not be separated. Nonetheless, this supports a clear practical conclusion that diversification is most valuable when it creates a well-established, resource-efficient crop stand and is combined with steady control of perennial weeds.

Future research should combine long-term rotation trails, crop-specific sole-crop controls, and several planting densities with farmer-led testing and advisory support because this outcome depends on how diversification is designed and adapted to local conditions. This would help turn treatment-specific findings into practical guidance on crop combinations, establishment, and perennial-weed management. Policy should reinforce the same approach. Common Agricultural Policy eco-schemes can reduce some of the financial risk of adopting sustainable practices, but incentives should reward useful agronomic functions such as competitive crop cover, efficient resource use, and durable weed suppression, rather than species numbers alone (European Parliament & Council of the European Union, 2021). Linking evidence, farm-level knowledge, and targeted support would make crop diversification more reliable in organic arable systems.

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This is to us!