Intercropping Legumes with Winter Oilseed Rape: A Strategy for Improved Weed Control, High Biomass and Enhanced Nitrogen and Chlorophyll concentration.

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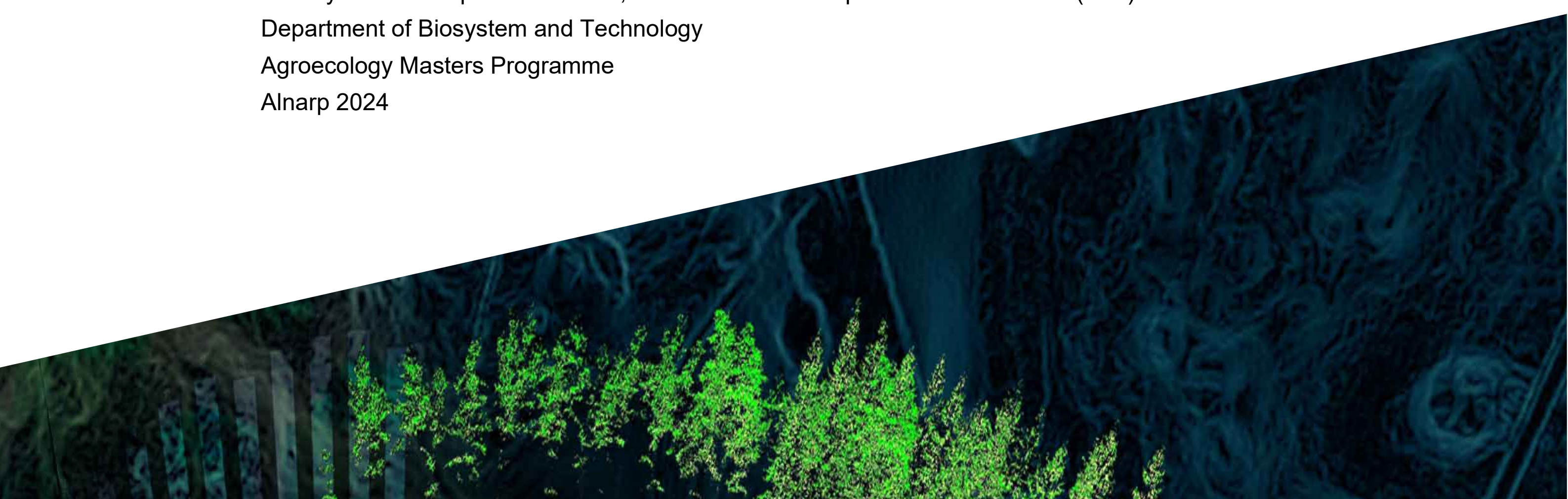
Degree project • 30 credits
Swedish University of Agricultural Sciences, SLU
Faculty of Landscape Architecture, Horticulture and Crop Production Science (LTV)
Department of Biosystem and Technology
Agroecology Masters Programme
Alnarp 2024



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Credits: 30 credits
Level: 2nd cycle A2E
Course title: **Independent Project in Agricultural science, A2E - Agroecology Master´s Programme**
Course code: **EX0848**
Programme/education: Agroecology - Master´s Programme
Course coordinating dept: Department of Biosystem and Technology
Place of publication: Alnarp
Year of publication: 2025
Keywords: Winter Oilseed Rape (WOSR), Legume Intercropping, Chlorophyll Content, Weed Biomass, Nitrogen Fixation, Agroecological Practices

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Abstract

Intercropping legumes with Winter Oilseed Rape (WOSR) presents a viable agro-ecological strategy to mitigate issues in monoculture systems, including insufficient weed control, overdependence on synthetic nitrogen fertilizers, and diminished crop biodiversity. While intercropping with cereals and legumes is extensively documented for its agronomic and ecological advantages, such as enhanced resource use efficiency, soil health, and production, the intercropping of winter oilseed rape with legumes is still inadequately investigated. This study examines the impact of several WOSR-legume intercropping combinations on crop growth, productivity, nitrogen and chlorophyll content and also weed assessment. The experiment was performed at the Lönnstorp research station of the Swedish University of Agricultural Sciences (SLU), with 24 intercropping treatments replicated across four plots utilizing a Randomized Complete Block Design (RCBD). Weed coverage percentages were determined for all 24 treatments while chlorophyll content was determined for 20 treatments. Furthermore, measurement of dry biomass weight and nitrogen content in WOSR, legumes, weeds were estimated in 13 treatments. The study revealed that intercropping WOSR with over-wintering peas under reduced N input, 50 cm wide row spacing between and 25% sowing density of winter peas planted six weeks after WOSR, had the highest leaf chlorophyll content and indicate less weed density. Moreover, the intercropping treatments improved nitrogen and chlorophyll content compared with sole crop winter oilseed rape (WOSR). Notably, WOSR intercropped with over-wintering faba bean using reduced N input with 50 cm wide row spacing and 50% faba bean sowing density sown 10 days after WOSR produced the highest legume biomass and nitrogen content. These results show that intercropping can reduce the use of synthetic fertilizers and herbicides, improve weed suppression, and maximize nitrogen use efficiency. It has the potential to fosters sustainable agriculture by enhancing the quality of the soil and avoiding negative impacts on the environment.

Keywords: Agroecological Practices, Chlorophyll Content, Nitrogen Fixation, Legume Intercropping, Winter Oilseed Rape (WOSR), Weed Biomass

Foreword

Agriculture is at a crossroads where the need to feed the increasing population meets the need to feed the population sustainably. Specialized monoculture and sole cropping systems that have been adopted in the modern world as a means of increasing production in the short run have brought about serious ecological problems such as nutrient depletion, pest resistance, and loss of biodiversity. These challenges are worse in crops like WOSR which require a lot of input and are very sensitive to competition from weeds and nutrient deficiencies.

Intercropping, which has been used for centuries, has returned to the field as a new approach to agroecology. There is therefore the need for introducing legumes into WOSR systems in order to benefit from such features as biological nitrogen fixation and improved and weed-free soil conditions, among other aspects that reduce the dependence of farmers on synthetic fertilizers. This study examines these possibilities with emphasis on the interaction between WOSR and legume species under different management regimes.

The insights presented in this work are the culmination of rigorous field experiments and analyses conducted at the Swedish University of Agricultural Sciences. Beyond their immediate applicability to WOSR-legume systems, the findings contribute to the broader discourse on sustainable agriculture and resource-efficient cropping systems.

I am deeply grateful to my supervisor, academic mentors, and peers who supported this endeavor. I also acknowledge the Swedish University of Agricultural Sciences for providing a conducive environment for research and the SITES research station for facilitating the fieldwork. I hope this research inspires further exploration of agroecological practices and their potential to transform agriculture for a more sustainable future.

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Abbreviations

WOSR - Winter Oilseed Rape

RCBD - Randomized Complete Block Design

SLU - Swedish University of Agricultural Sciences

BNF - Biological Nitrogen Fixation

ANOVA - Analysis of Variance

LSD - Least Significant Difference

EPA - Elemental Particle Analyser

WP - Over-wintering Pea

FA - Frost-sensitive Faba Bean

CV - Coefficient of Variation

N - Nitrogen

C - Carbon

HSD - Honest Significant Difference (Tukey's Test)

SPSS - Statistical Package for the Social Sciences

GC - Gas Chromatography

INTRODUCTION

Modern agriculture has significant challenges arising from the combined necessity of nourishing an expanding global populace while preserving environmental integrity. Traditional cropping systems, which typically prioritize the cultivation of a single crop species in a specific region for operational efficiency and production maximization, have resulted in various ecological and agronomic challenges. Such systems often requires high applications of chemical fertilizers, pesticides, and water, which reduce capacity hamper the ecosystem, and pollute the ground in the long run (Rashmi et al. 2020). However, these methods show low diversification, making them vulnerable to pests, diseases, and climate change impacts. These are compounded by limited resources and unpredictable effects of global climate change, which call for new approaches to farming. Intercropping, growing different crops in the same field at one time, is a more realistic approach because it ensures proper utilization of resources in a system as well as strengthens the structure of the existing farming systems so that sustainability becomes an achievable goal in farming sector (Vikas and Ranjan 2024).

Monoculture refers to the practice of growing a single plant species in a given field in different seasons of farming. It is used often for its simplicity and ability to handle yields, even though it often causes numerous problems. In monoculture systems, the crops are vulnerable to shocks such as drought, floods, and temperature changes since the code has little genetic variation in the plants it produces. Reduced gene differentiation makes crops more susceptible to attack by pests and diseases since the system, by its design, lacks the capacity for self-healing (Lin 2011). Hence, it is often costly and environmentally undesirable to maintain crop yields in monoculture practices, which often require investment in water, fertilizers, and pesticides (Nguyen et al. 2022; Belete & Yadete 2023). It gives prominence to the limitations of monoculture strategies and reaffirms the need for a stronger and diverse practice of agriculture.

The existing production systems such monoculture on large farms and intensive use of inputs especially fertilizers and pesticides are harmful to soil quality, biodiversity, and the environment. Excessive application of chemical fertilizers depletes the essential nutrients and a continuous use of pesticide causes pest resistance and hence require even more chemical (Latvala et al. 2021; Kole et al. 2019).

Winter oilseed rape (WOSR, *Brassica napus*), also known as canola, is an important oilseed crop with high-quality oil and meal production in Sweden and many countries around the globe. However, the cultivation of WOSR, while essential, presents significant challenges, particularly when grown as a monocrop. WOSR is a poor competitor with weeds, especially at the early growth stage, and requires frequent weed control measures that include the use of herbicides (Jeromela et al. 2017). In this case, excessive use of herbicides brings about the development of new weed types that are even more challenging to control hence in the process of weed control, more herbicides are used and the whole process becomes a vicious cycle. Lastly, WOSR is also highly vulnerable to several pests and diseases including; blackleg, *sclerotinia* stem rot, and light leaf spot pests that can decrease yields greatly if not controlled (Fortune 2022). Other important pests that affect WOSR production include pollen beetles (*Meligethes* spp.) and cabbage stem flea beetles (*Psylliodes chrysocephala*) (Jeromela et al. 2017). Another major constraint for WOSR cultivation is nutrient management. The crop needs a lot of nitrogen to grow but the use of synthetic nitrogen fertilizers leads to increased production costs and environmental problems like water pollution and emission of greenhouse gases (Liu et al. 2021). Climate change is also a threat to WOSR production; temperature changes and drought stress affect flowering and seed formation, decreasing the yield quantity and quality (Petkova et al. 2019). Based on these threats, there is a need for developing a more sustainable and resilient production systems for WOSR cultivation.

1.2. Aim of the research

The objective of this study is to assess the effect of different WOSR legume intercropping designs on crop biomass, weed assessment, nitrogen and chlorophyll contents. The study will answer the following specific research questions.

- How does intercropping influence crop biomass, weed competitiveness, nitrogen and chlorophyll content in WOSR and legume intercrop?

- What are the differences between WOSR-legume intercrops and WOSR sole crop in terms of nitrogen, chlorophyll content, weed estimation and crop biomass?

Recent studies have pointed to the possibility of using alternative cropping systems to overcome the problems associated with monocultures and sole crops. Among these, intercropping has attracted more attention as a result of showing positive effects on resource use efficiency, avoidance of synthetic inputs, and higher levels of crop resistance (Brooker et al. 2015). Such benefits stem from the uninterruptible relation of crop interactions that enhance element recycling and environmental homeostasis (Yang et al. 2023).

Although the recognized benefits of intercropping, significant knowledge gaps persist concerning the specifics of intercropping diverse crops, particularly the impacts of various crop combinations under differing environmental conditions. The effects of intercropping winter oilseed rape with different legume species, especially frost-sensitive legumes, exhibit considerable potential yet remain inadequately investigated. A study by Cadeaux et al. (2015) conducted in France focused on a limited number of frost-sensitive legume species grown with WOSR. This study mainly focused on the effects of these specific legume-WOSR combinations on nitrogen fixation, weed suppression, and crop productivity. The intercropping treatments in Cadeaux's study were not very diverse, as the author only examined a few species of legumes within a few controlled conditions. A number of additional studies have looked into WOSR-legume intercropping in addition to Cadeaux's. For instance, Emery et al. (2021) stated that intercropping oil seed rape (OSR) with legumes offer multifunctional crop protection. Their study found that oil seed rape intercropped with legumes reduced weed biomass, decreased cabbage stem flea beetle oviposition, and did not elevate slug and pathogen damage, indicating species specific benefits for pest and weed control. On the other hand, Dayoub et al. (2022) also studied that intercropping of OSR with legumes reduced the weed infestation and improved the oil seed rape crop productivity, again with species specific complementarity and replacement design playing key roles.

Our study examined 24 treatments that combined mechanical hoeing with varying row distances (narrow and wide) and high and low nitrogen fertilization applications, encompassing both frost-sensitive and frost-tolerant legume varieties. Furthermore, chlorophyll content and nitrogen percentage were evaluated, and their association was analyzed to determine their impact on quality. Additionally, weed evaluation and dry biomass measurements were conducted to analyze crop biomass and to investigate whether which combinations of WOSR with legumes have enhanced overall crop productivity under the climatic conditions of Scandinavia. This diversity allows for a better understanding of the interdependence between different crops, focusing on nitrogen in winter oilseed rape and legumes. In addition, while Cadeaux's study was based on nitrogen fixation as the primary ecological benefit, the present study provides a broader analysis, including nitrogen and chlorophyll concentrations and measurement of dry weight biomass and weed assessment under various management practices and different combinations of WOSR and legumes.

The current study aims to fill the knowledge gaps in above mentioned studies, by assessing the feasibility of intercropping WOSR with legumes with the view of improving sustainability and production. The rationale for this study is anchored on the rising need to develop cropping systems that can meet the two objectives of enhancing crop yields and the impacts on the environment. This research seeks to improve nitrogen content, and resource use efficiency in WOSR cropping systems through legume integration. The expected benefits of such systems include reduced use of synthetic nitrogen fertilizers, improved weed and biomass as well as nitrogen and chlorophyll content.

BACKGROUND INFORMATION

Agroecology, as a holistic approach to farming, integrates ecological principles with sustainable agricultural practices to promote food systems that are resilient, environmentally sound, and economically viable. For example, winter cereal cover crops are grown in part to suppress weed growth, but more importantly to enhance soil and environmental quality. It is also a key element of agroecology that agriculture must be made more environmentally sustainable through the adoption of practices like crop rotation, intercropping, and organic farming. This study is based on the premise that sustainable farming systems development goes beyond merely increasing agricultural output. It encompasses measures that improve soil and ecological health as well as support diversity.

Our research activity relates to a specific topic within agroecology, i.e., intercropping systems, which seek to enhance crop production with reduced environmental disruptions. We work on intercropping to improve nitrogen and chlorophyll content and contribute to the agroecological principles of designing improved farming systems that are more productive, less weedy, and more ecologically sustainable. Integrating ecofarming and agriculture in this manner corresponds to the agroecological goal of developing food systems that are productive and environmentally sustainable (Altieri 2018; Tittonell 2020).

2.1. Weed suppression mechanisms in intercropping systems

It has been established that intercropping is effective in the suppression of the growth of weeds. An example is where crops are made to compete for light, water, and nutrients to grow. Additionally, Stomph et al. (2020) suggest that intercropping involves growing plants with diverse resource requirements and growth patterns which will make them compete more aggressively with the pests on the same farm. Therefore, intercropping systems can employ allelopathy to control weeds. Allelopathic interaction is a process in which one plant affects the growth or development of other plants in the vicinity of the releasing plant (Choudhary et al. 2023). Moreover, some allelochemicals released by the crops through their microorganisms can induce weed dormancy and also suppress their germination and seedling growth (Xiao et al. 2020). All in all, we can consider that such interactions between plants because of allelopathy could be used as ways to combat unwanted plants in our ecosystems.

In intercropping systems, other methods of weed control include mechanical and climatic methods. This may result in a dense canopy since some crops may shade out the others while some crops may prevent access to sunlight by their weed competitors (Biswas et al. 2023). Likewise, alteration of soil temperature, moisture, or microbial status may render conditions unfavorable for the emergence and early growth of annual broad-leaved weeds such as Chenopodium album L that are common in agricultural fields that need a high level of light penetration through the soil surface before they start their above-ground vegetative growth phase (Liebman & Staver 2001). Also useful are those intercropping systems which act hosts beneficial organisms involved in biological control agents against undesired plant species while also enhancing biodiversity at large. There are certain more diverse plantings which will attract diseases and herbivores that act predators on these particular types weeds thereby reducing their populations within such farming systems (Brooker et al. 2015). Legumes can also improve soil fertility, crops growth enhancement as well as improve crops ability to compete with weeds in intercropping systems.

2.2. Legume-cereal facilitation and growth promotion

Legume-cereal intercropping systems have generated a lot of interest as they can promote growth and facilitate interactions. Nitrogen transfer from legume to cereal crop is one of main mechanisms supporting this facilitation (Kocira et al. 2020). Legumes and rhizobia bacteria work in collaboration with each other in symbiosis to fix atmospheric nitrogen which can then be utilized by legumes to improve the growth and productivity of their co-occurring cereal crops (Kebede 2021). Another way that enhances growth in legume-cereal intercropping systems is the complementary use of resources. Sometimes, legumes and cereals have different root patterns, nutrient requirements and resource acquisition strategies (Brooker et al. 2015). When grown together, however, such an arrangement allows them access to a broader range of resources and markets thereby increasing overall resource efficiency while reducing competition for scarce resources.

In addition, there are different soil processes that intercropping systems can encourage for healthy plants. Soil fertility can also be improved by legume through increasing the organic matter content, nitrogen fixation, and improving soil structure (Chamkhi et al. 2022). Additionally, diversification of plant population that occurs when using intercropping system supports a more active and diverse soil microbial community critical for nutrient cycling and promoting plant growth. Furthermore, intercropped legumes like beans and cereals could help systems to become more robust in response to environmental stressors such as heat, pest pressure or drought (Stomph et al. 2020). The facilitative interactions of this diverse plant community can enhance temperature regulation, water use efficiency and biotic/abiotic resistance leading to overall growth promotion and yield stability (Enebe et al. 2018).

2.3. Intercropping and disease management in oilseed crops

Various cropping systems with oilseed crops have shown promise in the management of diseases among different crop species. Modification of microclimate is the key cause for pathogen reduction in intercropped fields (Boudreau 2013). Intercropping with multiple crop species can alter humidity, temperature and air movement creating conditions unsuitable for some plant diseases to develop and spread (Kaur et al. 2021). Non-host plants could provide important physical barriers that may disturb the regularity of host populations interrupting disease transmission and dissemination. Also, increasing plant diversity within intercrops can aid disease control. For example, an intercrop system designed to reduce illness might also support beneficial species like pathogens' natural enemies or antagonistic bacteria (Huss et al. 2022). This diverse community of plants can supply these useful organisms with resources or habitats which then increases their numbers as well as activity levels (Hartmann et al. 2023).

Moreover, it is evident that through systemic resistance induction intercropping systems contribute towards protecting against infections among plants (Boudreau, 2013). Interactions between different crops along with their associated microorganisms could trigger the synthesis of antimicrobial compounds as well as activation of diverse resistance mechanisms that prompt defense responses in plants (Zehra et al. 2021).

2.4. Nitrogen fixation and transfer in legume-based systems

Legume-based intercropping systems have attracted attention because they can fix nitrogen from the air by the help of rhizobia bacteria (Lai et al. 2022). Another crop which is non-leguminous and a legume crop may share in this biological nitrogen fixation process through making additional soil nitrogen available to them (Kebede 2021).

Nitrogen fixed by legumes may be transferred to non-legume crops through several ways. One way includes releasing compounds of nitrogen during decomposition of legume residues that can then be taken up by associated plants (Kebede 2021). Furthermore, mycorrhizal networks, exudates from roots and leakages could allow direct utilization of fixed N by non-nodulating species (Reay et al. 2022). Also relevant are types of legumes used, soil characteristics such as fertility status or pH levels, environmental factors like temperature variations and specific design options for mixed cropping systems with different plant arrangements among other factors all affect how much nitrogen is fixed and transferred within legume-based systems (Lai et al. 2022). Additionally, the efficiency of BNF and subsequent N-transport may be influenced by the moisture content in subsoil depending on the presence or absence of suitable rhizobia strains and moisture in root zone surface area where these two symbiotic organisms coexist (Soumare et al. 2020).

The effectiveness with which nitrogen fixation occurs during its subsequent translocation largely determines whether the agro-practices associated with leguminous intercropping are sustainable. Mono-cropping methods can help mitigate greenhouse gas emissions while decreasing pollution due to synthetic fertilizers as well as save more from synthetic N inputs into farm fields (Kebede 2021). In like manner, higher supplies of nitrogen enhance system resilience in general besides boosting crop productivity and nutritional quality for harvested produce (Mahmud et al. 2021).

MATERIALS AND METHODS

3.1. Location and climatic conditions

The current study was conducted at the SITES, Lönnstorp research station of the Swedish University of Agricultural Sciences (SLU), situated in Scania Province, southern Sweden at 55°39'N, 13°19'E. Lönnstorp climate is of temperate oceanic type owing to its location between the Baltic Sea. This region is suitable for intercropping research of legumes with winter oilseed rape because of its favorable climatic and soil conditions.

3.2. Experimental Design and Treatments

The experimental design includes 24 treatments, of which 20 were selected to assess chlorophyll content, while all 24 were utilized for evaluating weed coverage percentage. WOSR, winter pea (wp), faba beans (wfa) were planted as sole crops respectively and intercrops in the summer-autumn of 2023, with assessments carried out in January 2024. With the exception of clover, which was exclusively planted as a WOSR intercrop. Furthermore, 13 treatments were selected for the investigation of nitrogen content, along with dry biomass measurement.

The purpose of this study was also to check how various mechanical weed management methods affected winter oilseed rape (WOSR) in terms of weed management as well as crop yields, focusing on both weed suppression and crop performance. All 24 treatments were assessed (except frost sensitive and WOSR sole), each characterized by specific combinations of autumn mechanical weed control interventions and spring (If needed). Autumn treatments included hoeing about 10 days after sowing or harrowing before sowing, and spring treatments involved mostly hoeing at various crop development stages. Certain treatments consisted of repeated hoeing at 10 and 42 days after sowing, while others involved single or no weeding applications. The experiment aimed to determine the effectiveness of these strategies in reducing weed competition and promoting optimal crop development.

Table 1. Mechanical weed control strategies on winter oilseed rape (WOSR) across autumn and spring seasons.

Treatments No.	Labels	Treatments	Autumn Mechanical Weed Control	Spring Mechanical Weed Control
1	P	WOSR+wp hi w 50	hoeing ca 10 days post-sowing	-
2	V	wfa del	harrow shortly before sowing	-
3	K	WOSR+wfa hi w 50	hoeing ca 10 days post-sowing	-
4	I	WOSR+fs fa lo w	-	hoeing
5	R	WOSR+wp lo w 50 del	hoeing twice, 10 and 42 days post-sowing	-
6	T	WOSR+wp lo w 25 del	hoeing twice, 10 and 42 days post-sowing	-
7	A	WOSR hi n	-	-

8	U	wfa	harrow shortly before sowing	-
9	X	wp del	harrow shortly before sowing	-
10	G	WOSR+fs cl lo w del	hoeing ca 10 days post-sowing	hoeing
11	F	WOSR+fs cl lo w	-	hoeing
12	B	WOSR hi w	-	-
13	E	WOSR+fs cl lo n	-	-
14	Q	WOSR+wp lo w 50	hoeing ca 10 days post-sowing	-
15	C	WOSR lo w	hoeing ca 10 days post-sowing	hoeing
16	W	wp	harrow shortly before sowing	-
17	D	WOSR+fs cl hi n	-	-
18	O	WOSR+wfa lo w 25 del	hoeing twice, 10 and 42 days post-sowing	-
19	L	WOSR+wfa lo w 50	hoeing ca 10 days post-sowing	-
20	J	WOSR+fs fa lo w del	hoeing ca 10 days post-sowing	hoeing
21	S	WOSR+wp lo w 25	hoeing ca 10 days post-sowing	-
22	H	WOSR+fs fa hi w	-	-
23	N	WOSR+wfa lo w 25	hoeing ca 10 days post-sowing	-
24	M	WOSR+wfa lo w 50 del	hoeing twice, 10 and 42 days post-sowing	-

Table 2. Abbreviations and Spacing Descriptions of the 24 Treatments:

Treatment abbreviation	**input level**	**row spacing**	**Treatment description**
WOSR+wp hi w 50	high	Wide	WOSR intercropped with over-wintering pea (wp), high input levels, wide row spacing, 50% sowing density of wp sown ten days after WOSR
wfa del	low	Narrow	Over-wintering faba bean sole crop, no N fertilization or pesticides, wfa sown six weeks later than WOSR
WOSR+wfa hi w 50	high	Wide	WOSR intercropped with over-wintering faba bean (wfa), high input levels, wide row spacing, 50% sowing density of wfa sown ten days after WOSR
WOSR+fs fa lo w	low	Wide	WOSR intercropped with frost-sensitive faba bean, reduced inputs, wide row spacing, fs fa sown on same date as WOSR

WOSR+wp lo w 50 del	low	Wide	WOSR intercropped with over-wintering pea, reduced inputs, wide row spacing, 50% sowing density of wp sown six weeks after WOSR
WOSR+wp lo w 25 del	low	Wide	WOSR intercropped with over-wintering pea, reduced inputs, wide row spacing, 25% sowing density of wp sown six weeks after WOSR
WOSR hi n	high	Narrow	WOSR sole crop, high input levels (hi; full rates of N fertilization and pesticides), narrow row spacing (n; 12.5 cm)
Wfa	low	Narrow	Over-wintering faba bean sole crop, no N fertilization or pesticides, wfa sown ten days later than WOSR
wp del	low	Narrow	Over-wintering pea sole crop, no N fertilization or pesticides, wp sown six weeks later than WOSR
WOSR+fs cl lo w del	low	Wide	WOSR intercropped with frost-sensitive clover, reduced inputs, wide row spacing, fs cl sown ten days after WOSR
WOSR+fs cl lo w	low	Wide	WOSR intercropped with frost-sensitive clover, reduced inputs, wide row spacing, fs cl sown on same date as WOSR
WOSR hi w	high	Wide	WOSR sole crop, high input levels wide row spacing (w; 50 cm)
WOSR+fs cl lo n	low	Narrow	WOSR intercropped with frost-sensitive clover, reduced inputs, narrow row spacing, fs cl sown on same date as WOSR
WOSR+wp lo w 50	low	Wide	WOSR intercropped with over-wintering pea, reduced inputs, wide row spacing, 50% sowing density of wp sown ten days after WOSR
WOSR lo w	low	Wide	WOSR sole crop, reduced inputs (lo; 25% reduced N fertilization, no pesticides), wide row spacing
Wp	low	Narrow	Over-wintering pea sole crop, no N fertilization or pesticides, wp sown ten days later than WOSR
WOSR+fs cl hi n	high	Narrow	WOSR intercropped with frost-sensitive clover (fs cl), high input levels, narrow row spacing, fs cl sown on same date as WOSR
WOSR+wfa lo w 25 del	low	Wide	WOSR intercropped with over-wintering faba bean, reduced inputs, wide row spacing, 25% sowing density of wfa sown six weeks after WOSR
WOSR+wfa lo w 50	low	Wide	WOSR intercropped with over-wintering faba bean, reduced inputs, wide row spacing, 50% sowing density of wfa sown ten days after WOSR
WOSR+fs fa lo w del	low	Wide	WOSR intercropped with frost-sensitive faba bean, reduced inputs, wide row spacing, fs fa sown ten days after WOSR
WOSR+wp lo w 25	Low	Wide	WOSR intercropped with over-wintering pea, reduced inputs, wide row spacing, 25% sowing density of wp sown ten days after WOSR
WOSR+fs fa hi w	high	Wide	WOSR intercropped with frost-sensitive faba bean (fs fa), high input levels, wide row spacing, fs fa sown on same date as WOSR

WOSR+wfa lo w 25	Low	Wide	WOSR intercropped with over-wintering faba bean, reduced inputs, wide row spacing, 25% sowing density of wfa sown ten days after WOSR
WOSR+wfa lo w 50 del	Low	Wide	WOSR intercropped with over-wintering faba bean, reduced inputs, wide row spacing, 50% sowing density of wfa sown six weeks after WOSR

Table 3. Abbreviations and Spacing Descriptions of the 13 Treatments Selected for Dry biomass weight and Nitrogen content measurement:

Treatment abbreviation	Treatment Description
WOSR+wfa lo w 50	WOSR intercropped with over-wintering faba bean, reduced inputs, wide row spacing, 50% sowing density of wfa sown ten days after WOSR
Wfa	Over-wintering faba bean sole crop, no N fertilization or pesticides, wfa sown ten days later than WOSR
WOSR+fs fa lo w	WOSR intercropped with frost-sensitive faba bean, reduced inputs, wide row spacing, fs fa sown on same date as WOSR
WOSR+wp lo w 50	WOSR intercropped with over-wintering pea, reduced inputs, wide row spacing, 50% sowing density of wp sown ten days after WOSR
WOSR+wfa lo w 25	WOSR intercropped with over-wintering faba bean, reduced inputs, wide row spacing, 25% sowing density of wfa sown ten days after WOSR
WOSR hi w	WOSR sole crop, high input levels wide row spacing (w; 50 cm)
WOSR+wp lo w 25	WOSR intercropped with over-wintering pea, reduced inputs, wide row spacing, 25% sowing density of wp sown ten days after WOSR
WOSR+wfa hi w 50	WOSR intercropped with over-wintering faba bean (wfa), high input levels, wide row spacing, 50% sowing density of wfa sown ten days after WOSR
Wp	Over-wintering pea sole crop, no N fertilization or pesticides, wp sown ten days later than WOSR
WOSR lo w	WOSR sole crop, reduced inputs (lo; 25% reduced N fertilization, no pesticides), wide row spacing
WOSR hi n	WOSR sole crop, high input levels (hi; full rates of N fertilization and pesticides), narrow row spacing (n; 12.5 cm)
WOSR+fs fa hi w	WOSR intercropped with frost-sensitive faba bean (fs fa), high input levels, wide row spacing, fs fa sown on same date as WOSR
WOSR+wp hi w 50	WOSR intercropped with over-wintering pea (wp), high input levels, wide row spacing, 50% sowing density of wp sown ten days after WOSR

Table 4. Nitrogen Levels, Fertilizer Type, and Plant Nutrition in the Experimental Treatments:

Crop	Treatment	Nitrogen Application	Fertilizer Type	Explanation
WOSR (Winter Oilseed Rape)	High Input (hi)	Full rate (e.g., 53 kg N in autumn)	Yara Raps (N + S)	High nitrogen promotes vegetative growth, maximizes yield; sulfur aids oil formation and protein synthesis.
WOSR (Winter Oilseed Rape)	Reduced Input (lo)	75% of full rate (e.g., 40 kg N in autumn)	Yara Raps (N + S)	Lower nitrogen reduces cost and environmental impact, while maintaining sulfur for essential functions.
Companion Crops (wfa, wp)	U (wfa)	None	PKS (no N)	PKS provides phosphorus, potassium, and sulfur; no nitrogen to avoid inhibiting nitrogen fixation.
Companion Crops (wfa, wp)	V (wfa del)	None	PKS (no N)	Similar to U, for overwintering faba beans.
Companion Crops (wfa, wp)	W (wp)	None	PKS (no N)	PKS supports nutrient needs for overwintering peas without adding nitrogen.
Companion Crops (wfa, wp)	X (wp del)	None	PKS (no N)	Same as W, with a focus on nutrient balance.

i) **Fertilizer Application:** The treatments are distinguished based on two levels of nitrogen application: high (hi) and low (lo). For instance, treatments are named as "WOSR hi n" which means that the treatment has high nitrogen level (53 kg) which is essential for the betterment of crop yield and health of the plants, whereas "lo n" is the treatment where uses low nitrogen input (40 kg) to analyse the effect of less fertilization on crop growth and yield. In companion crops, such as winter pea and faba bean, PKS (phosphorus, potassium, and sulfur) fertilizer is utilized as it supplies critical nutrients—phosphorus, potassium, and sulfur—without nitrogen, hence facilitating effective nitrogen fixation by the legumes. This prevents the inhibition of the biological nitrogen fixation process. Conversely, the WOSR and WOSR-legume intercropping treatments utilize Yara Raps, which contains both nitrogen and sulfur. This formulation addresses the elevated nitrogen requirements of WOSR, enhancing vegetative development and optimizing output, while sulfur facilitates oil production and protein synthesis in the crop.

ii) **Plant Spacing:** Spacing is also an important aspect in these treatments with configurations varying from wide (w) to narrow (n). The exact distances for these row spacing types are "Narrow spacing (12.5 cm) and wide spacing (50 cm).

iii) **Legume Inclusion:** The study consisted of intercropping WOSR with frost-sensitive legumes (e.g., frost-sensitive faba bean and clover 'Alexandrin') and frost-tolerant/winter-hardy legumes (e.g., winter-hardy faba bean and peas). This approach aimed to assess how these legumes can support WOSR growth and reduce the need for synthetic fertilizers through biological nitrogen fixation.

3.3. Design and Randomization

This field experiment utilized a randomized complete block design (RCBD) to assess the effects of various treatments, with a total of 24 treatments each replicated four times. This design is particularly useful in controlling for field heterogeneity and environmental gradients, increasing the statistical efficiency of the results and allowing for more accurate comparisons between treatments (Gomez & Gomez 1984).

The plots (6 x 15 m) were then laid out in a randomized complete block design (RCBD) to reduce variability in the field. This arrangement required appropriate consideration of the plot size, row spacing, and planting densities to offer the right growing conditions for WOSR and the inter-sown legumes.

Further, other essential weed management practices that are associated with planting practices were undertaken to ensure that a good weed-free environment was created before planting through plowing and harrowing of the field. The land was tilted for the improvement of the structure of the soil with the aim of increasing its water-holding capacity which is important for the discussed crops. This was done in order to optimize the use of the resources and establish the environment for the investigation of the benefits of intercropping in relation to agronomic factors.

Replicability: Each treatment is applied within blocks so that the effects of the treatments can be statistically compared with the field while accounting for variation. The alignment of the blocks and treatments within the blocks is presented in Appendix (Tables 28 and 29).

3.4. Data Collection

Chlorophyll content data and weed coverage percentages were recorded over three consecutive days, March 5th, 6th, and 7th. For dry weight analysis, plants were uprooted over two days, April 2nd and 3rd. The collected material was dried in an dry machine at 60°C for approximately 48 hours (April 3rd to 5th) and weighed on April 6th. The milling of the dried plants was carried out on April 20th or 21st, followed by weighing and preparing powdered samples. Finally, the powdered samples were encapsulated (5.5 g each) for nitrogen (N) analysis on April 29th and 30th.

3.4.1. Chlorophyll Contents

The level of chlorophyll defined how nourished plants were and their readiness for photosynthesis so it was quite suitable. This is because of the use of leaf absorption features that are associated with relative chlorophyll concentration (Kalaji et al., 2017). For chlorophyll content determination, a systematic sampling technique was applied in each sample plot. In particular, three 0.25 sq.m areas were chosen in each plot (in total 0.75 sq.m) to represent the plots and to provide the most accurate and consistent samples. In the selected areas, two upper and two lower WOSR leaves were randomly selected from three plants to measure chlorophyll content to eliminate any bias arising from leaf choice. Chlorophyll content in CCI unit was then measured using the Apogee chlorophyll meter, a tool that is used to measure chlorophyll content in leaves without damaging the tissue. This methodology makes it possible to obtain accurate data on chlorophyll content and at the same time, to indicate fluctuations within the plot, but at the same time, to adhere to the general methodology for all samples.

3.4.2. Weed Cover

For the purpose of weed cover assessment, the same area as used for chlorophyll content measurement within each plot was used, a 0.25 sq.m area was marked in three different locations (total 0.75 sq.m) to ensure that the coverage was representative. The percentage of weed covering was then obtained by visually evaluating the weeds in the chosen sites; the related weed dry biomass was then measured and computed.

3.4.3. Plant Dry Matter Biomass

To determine plant biomass, plants including WOSR, legume and weeds, were collected following the same sampling area as used for chlorophyll content assessment. In each sample plot, randomly three selected plants were carefully uprooted (roots were washed to eradicate soil contamination) to avoid loss of biomass. Once collected, each plant sample was washed and then packed individually, labeled, and prepared for drying. The samples were placed in a drying machine for a period of three days at 60C to ensure complete moisture removal. After drying, the weight of each packet and the dried plant material was taken to determine the dry biomass. The total biomass of each plant sample was then determined by subtracting the weight of the empty packet from the total weight of the sample, thus giving a common unit of biomass for comparison between the samples.

3.4.4. Nitrogen Analysis

For the determination of nitrogen, the dried plant samples from the plant biomass estimation were used. The dried plant materials were then ground using a milling machine tofine powder in order to ensure uniformity in composition. From this powder, 5.5 grams were accurately weighed, placed in aluminum foil, and put in labeled tubes (same labelers used as shown in Table 1) to ensure safe carriage. These samples were then subjected to nitrogen analysis using the Elemental Particle Analyzer (EPA) standard method with the automatic autosampler using argon as carrier gas (Krotz et al. 2014). This method (also known as Dumus Nitrogen Analyzer) is accurate and reproducible in terms of quantifying nutrient content and is suitable for the assessment of nitrogen levels in the plant biomass.

i) EPA Method

The Flash 2000 Elemental Analyzer by Thermo Scientific also known as the Dumas Nitrogen Analyzer is an automated system that by the dynamic flash combustion method so special for nitrogen analysis. For analysis, 3 to 5 mg of each sample is weighed twice using the Mettler Toledo XP6 Microbalance with LabX software and placed in tin capsules. In operation, the precisely weighed sample is burned at high temperatures of 900 – 1000°C in the combustion chamber of the analyzer. The resulting gases are transported through a helium carrier to a second reactor containing copper, then through CO_2 and H_2O traps to a GC column with a thermal conductivity detector. GC separation also provides higher sensitivity compared to the purge and trap methods and thus quantification is possible.

Aspartic acid and acetanilide were used as calibration standards, with alfalfa (3.25% N) and Acetanilide R1 (10.36% N) as additional calibration standards.

3.5. Data handling and statistical analysis

Data from laboratory and field measurements were recorded and organized in Microsoft Excel. SPSS software was employed to conduct statistical analysis, specifically ANOVA to assess treatment effects, and Tukey's HSD test to compare group means for significant differences. Descriptive statistics, including means, standard deviations, and coefficients of variation, were calculated within each treatment to estimate variability. Data trends were visualized with tables, including bar graphs.

RESULTS

4.1. Chlorophyll level in the upper leaves:

The analysis of variance (ANOVA) for the chlorophyll content index (CCI) level in the upper leaves for 20 treatments are shown in table 5. The analysis shows that few treatments were significantly different from one another with $p \leq 0.05$.

Table 5. Analysis of variance among treatments for Chlorophyll level in the upper leaves

Source	DF	SS	MS	F	P
Replication	3	174.756	58.2521		
Treatment	19	694.246	36.5392	3.91	0.0000*
Error	56	523.042	9.3400		
Total	78				

* = Significant ($P \leq 0.05$), ns= Non-significant

Table 6. Chlorophyll content of the upper leaves of WOSR in different treatments. Treatments within the same homogenous group (sharing the same letter) are not significantly different.

Treatments	Mean (CCI) ± S.D	Homogenous groups
WOSR+ wp lo w 25 del	41.1±1.01	A
WOSR+ wp hi w 50	39.9±1.03	AB
WOSR+ wp lo w 50 del	38.7±0.49	ABC
WOSR with wp lo w 25	38.5±1.03	ABC
WOSR with wfa lo w 50 del	38.4±0.46	ABC
WOSR+ wp lo w 50	36.9±0.83	ABC
WOSR+ wfa hi w 50	36.1±0.64	ABC
WOSR hi n	34.5±0.99	ABC
WOSR+ fs cl lo w	34.3±1.43	ABC
WOSR with wfa lo w 25 del	34.3±0.95	ABC
WOSR hi w	34.1±0.80	ABC
WOSR with wfa lo w 25	34.1±0.62	ABC
WOSR lo w	33.5±0.85	ABC
WOSR+ fs cl lo w del	32.8±1.30	BC
WOSR with wfa lo w 50	32.5±1.17	BC
WOSR with fs fa hi w	32.1±0.55	BC
WOSR with fs fa lo w del	31.9±0.47	BC
WOSR+ fs cl hi n	31.3±1.37	BC
WOSR+ fs cl lo n	31.2±1.28	C
WOSR+ fs fa lo w	31.1±1.13	C

The chlorophyll content index (CCI) numerical values revealed distinct differences among the treatments, with the highest CCI value of (41.1) observed in the WOSR+ wp lo w 25 del treatment, significantly outperforming the other treatments which do not fall under the category of homogenous group A. This treatment, which involved wide-row distance and a specific nitrogen input, demonstrated a clear advantage in chlorophyll content. The treatments with similar CCI values, such as WOSR+ wp hi w 50 (39.9±1.03) and WOSR+ wp lo w 50 del (38.7±0.49), were grouped together under the same homogenous category (AB), showing no significant differences despite slight variations in row distance and nitrogen fertilizer application.

On the other hand, WOSR+ fs fa lo w, which had frost-sensitive legumes, had the lowest CCI value (31.1), significantly different from all other treatments which do not fall under the category of homogenous group C. This indicates that frost sensitivity in legumes, combined with lower nitrogen fixation capacity, likely limited the growth of WOSR in these treatments. Meanwhile, the frost-tolerant legumes (e.g., WOSR+ wp lo w 25 del) resulted in higher CCI values, suggesting that winter-hardiness and the ability to withstand frost conditions are key factors in optimizing chlorophyll content and overall crop performance. Also, the delayed planting of legumes probably enabled the WOSR plants to develop a more robust canopy and attain a more favorable growth phase prior to the escalation of competition for resources from the legumes. The comparison highlights the importance of selecting appropriate legumes—both in terms of frost tolerance and nitrogen-fixing capabilities—to achieve better outcomes in intercropping systems.

4.2. Chlorophyll level in the lower leaves

The analysis of variance (ANOVA) for the chlorophyll level in the lower leaves for 20 treatments are shown in table 7. Similar to the upper leaves, again few treatments were highly significantly distinctive from one another.

Table 7. Analysis of variance for Chlorophyll level in the lower leaves

Source	DF	SS	MS	F	P
Replication	3	17.750	5.9166		
Treatment	19	421.081	22.1622	2.99	0.0008*
Error	56	414.538	7.4025		

Total	78				

* = Significant (P≤0.05), ns= Non-significant

Table 8. Chlorophyll content in the lower leaves of WOSR. Treatments within the same homogenous group (sharing the same letter) are not significantly different.

Treatments	Mean (CCI) ± S.D	Homogenous groups
WOSR+wp lo w 25 del	31.2±0.91	A
WOSR+wp lo w 50 del	29.8±0.65	AB
WOSR+wp hi w 50	29.4±0.83	AB
WOSR with wfa lo w 50 del	29.2±0.59	AB
WOSR+wfa hi w 50	28.7±0.63	AB
WOSR+wp lo w 50	27.9±1.04	AB
WOSR with wp lo w 25	27.9±1.02	AB
WOSR hi w	27.3±1.13	AB
WOSR lo w	26.4±1.77	AB
WOSR with wfa lo w 50	26.2±0.95	AB
WOSR hi n	26.2±1.13	AB
WOSR with wfa lo w 25	26.1±0.76	AB
SWOSR+fs cl lo w del	25.3±1.07	AB
WOSR with wfa lo w 25 del	25.2±0.89	AB
WOSR+fs cl lo w	25.1±0.49	AB
WOSR+fs cl lo n	24.2±1.12	AB
WOSR with fs fa hi w	24.2±1.08	AB
WOSR+fs fa lo w	23.5±1.06	B
WOSR with fs fa lo w del	23.3±0.77	B
WOSR+fs cl hi n	22.9±0.66	B

In terms of comparison between treatments, the highest chlorophyll content in the lower leaves was found in the WOSR+ wp lo w 25 del treatment (31.2 CCI), which had the highest value for both upper and lower leaves, although the gap between the two leaves (upper and lower) was slightly more prominent in the lower leaves. Other treatments such as WOSR+ wp lo w 50 del (29.8 CCI) and WOSR+ wp hi w 50 (29.4 CCI) also showed higher chlorophyll levels in the lower leaves, which were relatively consistent with their performance in the upper leaves, falling into the same homogenous groups (AB) as the top-performing treatments in the upper leaves.

Conversely, the treatment WOSR+ fs cl hi n (22.9 CCI) had the lowest chlorophyll content in the lower leaves, consistent with its poor performance in the upper leaves. This treatment, along with WOSR+ fs fa lo w (23.5 CCI) and WOSR+ fs fa lo w del (23.3 CCI), also exhibited the lowest chlorophyll levels in both leaf sections, which suggests that the frost-sensitive legume components may be limiting the overall chlorophyll production in WOSR.

However, the delayed sowing of legumes, as shown in the WOSR+ wp lo w 25 del treatment, probably enabled the WOSR plants to develop a more robust canopy and enhanced photosynthetic efficiency prior to increasing competition with the legumes. This elucidates the elevated chlorophyll concentration in the lower leaves, as the plants effectively harnessed available resources for enhanced leaf development.

4.3. Chlorophyll level in upper leaves vs lower leaves

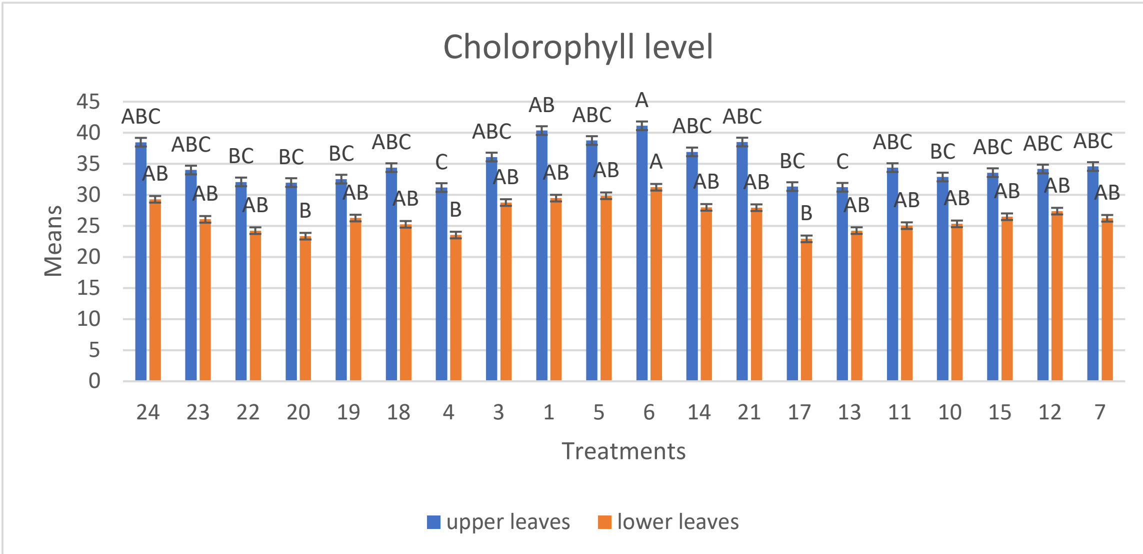

Figure 1. Chlorophyll level in upper leaves vs chlorophyll level in lowest leaves

The comparative examination of chlorophyll content in upper and lower leaves demonstrated notable trends across treatments. As seen in Figure 1, the chlorophyll content in the upper leaves ranged from (41.1 CCI) in the WOSR+ wp lo w 25 del treatment to (31.1 CCI) in the WOSR+ fs fa lo w treatment. In the lower leaves, the chlorophyll content ranged from (31.2 CCI) in WOSR+ wp lo w 25 del to (22.9 CCI) in WOSR+ fs cl hi n. This wide range in chlorophyll content highlights significant differences among the treatments, indicating that factors like legume type, frost sensitivity, and row distance play crucial roles in chlorophyll production.

The treatment WOSR+ wp lo w 25 del consistently performed the best, with the highest chlorophyll content in both the upper (41.1 CCI) and lower (31.2 CCI) leaves. This treatment, which includes a wide-row distance and reduced N inputs (40kg, i.e. 25% less than the high-input treatments with 53kg) appears to be highly beneficial for WOSR growth. The relatively high chlorophyll content suggests that this combination of factors is optimal for chlorophyll production and overall plant health.

In contrast, WOSR+ fs fa lo w showed the lowest chlorophyll content in the upper leaves (31.1 CCI), followed by WOSR+ fs cl hi n, which had the lowest chlorophyll content in the lower leaves (22.9 CCI). These treatments, involving frost-sensitive legumes, were significantly different from the higher-performing treatments in both leaf sections.

Other treatments, such as WOSR+ wp lo w 50 del (upper leaves: 38.7, lower leaves: 29.8) and WOSR+ wp hi w 50 (upper leaves: 39.9, lower leaves: 29.4) had moderate chlorophyll content. These treatments, which include wide-row distance and nitrogen application, did not show any differences in chlorophyll content between each other. This means that although these treatments are effective, they are not as effective as WOSR+ wp lo w 25 del.

However, all the treatments with frost-tolerant legumes (WOSR+ wp lo w 25 del, WOSR+ wp lo w 50 del) had significantly higher chlorophyll content in both the upper and lower leaves and it can be inferred that these legume types may be more beneficial for enhancing the plant growth and chlorophyll content. However, the addition of frost-sensitive legumes such as in WOSR+ fs fa lo w and WOSR+ fs cl hi n reduced chlorophyll content, suggesting that frost sensitivity may negatively affect WOSR chlorophyll synthesis and plant health.

One of the most interesting observations is the comparison of such treatments as WOSR+ wp lo w 25 del and WOSR+ wp lo w 50 del. Although both treatments showed relatively high chlorophyll levels in both leaf sections, WOSR+ wp lo w 25 del outperformed WOSR+ wp lo w 50 del, even in the lower leaves, despite both having a wide row distance. The treatment with a 25% seeding density of over-wintering pea likely exceeded the 50% density in chlorophyll content index due to reduced inter-plant competition. Decreased density of pea plants led to a more fair allocation of resources, such as sunlight, nutrients, and water, especially in wide row spacing, allowing both the upper and lower leaves of the WOSR to get adequate resources. The diminished pea density may have mitigated shadowing effects, hence improving photosynthesis in WOSR leaves. The six-week delay in pea planting enhanced the WOSR's root and leaf system growth, hence increasing its nutrient absorption capability. These ingredients jointly improved the chlorophyll content index in the following treatment. This result underscores the need of optimizing companion crop density for effective intercropping.

4.4. Weed cover Percentage

Less significant variations across all 24 treatments were shown by the analysis of variance (ANOVA) for weed cover ($p < 0.05$). The mean comparison test creates homogenous groups based on weed coverage and clarifies further the differences between treatment groups.

The results of the analysis of the variance were further subjected to the mean comparison test (LSD test) in table 10 to analyze the significant difference and the result of the least significant differences (LSD) test supported the results of the ANOVA. LSD test indicated that those treatments have the same alphabetic letters means that these treatments have the same results for weed coverage and showed non-significant results compared to each other. All treatments have significant differences among them.

Table 9. Analysis of variance for weed coverage percentage

Source	DF	SS	MS	F	P
Replication	3	7.11	2.3716		
Treatment	19	1024.60	53.9262	4.14	0.0000*
Error	56	729.38	13.0247		
Total	78				

* = Significant (P≤0.05), ns= Non-significant

Table 10. Response of treatments in weed cover. Treatments within the same homogenous group (sharing the same letter) are not significantly different.

Treatments	Means (%) ± S.D	Homogenous groups
WOSR+ fs cl lo n	28.7±0.76	A
WOSR+ fs cl hi n	27.9±1.12	AB
WOSR+fs cl lo w	26.2±1.14	ABC
WOSR with fs fa lo w del	24.9±1.12	ABC
WOSR+fs fa lo w	24.9±1.13	ABC
WOSR+fs cl lo w del	24.9±1.14	ABC
WOSR with fs fa hi w	24.5±1.06	ABCD
WOSR hi w	23.7±1.25	ABCD
WOSR with wfa lo w 50	23.3±0.95	ABCD
WOSR with wfa lo w 25	22.4±0.85	ABCD
WOSR lo w	21.2±1.49	ABCD
WOSR hi n	20.8±1.04	ABCD
WOSR with wfa lo w 25 del	19.9±0.98	ABCD
WOSR+wp lo w 50	19.9±1.11	ABCD

WOSR with wfa lo w 50 del	19.1±0.85	BCD
WOSR with wp lo w 25	18.7±1.13	BCD
WOSR+wp lo w 50 del	18.7±1.26	BCD
WOSR+wp hi w 50	17.8±1.14	BCD
WOSR+wfa hi w 50	17.1±1.15	CD
WOSR+wp lo w 25 del	15.4±0.95	D

According to Table 10, the treatments involving frost-sensitive legumes (fs) generally showed higher levels of weed coverage percentage compared to those with frost-tolerant legumes or those with wider row spacings. For example, WOSR+ fs cl lo n (28.7 %) had the highest level of weed coverage, followed by WOSR+ fs cl hi n (27.9 %), and WOSR+ fs cl lo w (26.2 %). These treatments, which incorporate frost-sensitive clover species (Alexandrin), exhibited a significant increase in weed presence, suggesting that frost-sensitive legumes may allow for more weed growth or less competition with weeds.

On the other hand, the treatments with frost-tolerant legumes, such as WOSR+ wp lo w 25 del (15.4 %), WOSR+ wp hi w 50 (17.8 %), and WOSR+ wfa hi w 50 (17.1 %), showed significantly lower levels of weed coverage. These results indicate that frost tolerance in legumes, along with row spacing and inter-row hoeing, may offer superior weed control. This is quite a contrast to the frost-sensitive treatments, which had higher weed growth, probably because of the dissimilar growth habits and competition of the two types of legumes.

The same pattern is observed when comparing different legume species, for example, frost-sensitive clover and faba bean (fs cl and fs fa) and other species like wp and wfa (frost-tolerant). The treatments with frost-tolerant legumes such as WOSR+ wp lo w 25 del and WOSR+ wp hi w 50 were significantly lower in weed coverage than the frost-sensitive legume treatments, which suggests that frost-tolerant legumes can be more effective competitors against weeds in the soil.

Furthermore, the treatments with wide row spacing such as WOSR+ wp lo w 25 del (15.4 %) and WOSR+ wp hi w 50 (17.8 %) had better weed suppression than the treatments with narrow row spacing such as WOSR+ fs cl lo n, which indicates that row spacing also has an influence on weed management. Since, small wider row spacing was combined with inter row-hoeing (during autumn season), this reduced the opportunities for weeds to grow.

In conclusion, frost-tolerant legumes, wide row spacing, and optimized legume species combinations seem to be more effective in controlling weed growth in WOSR systems, while frost-sensitive legumes contribute to higher weed coverage and may require additional management strategies for effective weed control.

4.5. Dry biomass weight of legumes

The analysis of variance (ANOVA) for dry weight for legumes under different treatments (10) found significant variation among the treatments (Table 11).

Table 11. Analysis of variance for legumes dry weight (g) under different treatments

Source	DF	SS	MS	F	P
Replication	3	0.00029	0.00010		
Treatment	12	0.15031	0.01253	143.69	0.0000*
Error	36	0.00314	0.00009		
Total	51	0.15373			

* = Significant (P≤0.05), ns= Non-significant

Table 12: Mean table for dry weight (g) of legumes

Treatment	Mean (g/0.75 sqm) ± S.D	Homogenous groups
Wosr with wfa lo w 50	0.21±0.02	A
Wosr+ fs fa lo w	0.04±0.02	C
Wosr with fs fa hi	0.02±0.01	C
Wp	0.16±0.02	A
Wfa	0.14±0.02	A
Wosr+ Wp hi w 50	0.12±0.02	B
Wosr+ wp lo w 50	0.01±0.01	C
Wosr with wfa lo w 25	0.05±0.02	C
Wosr with wp lo w 25	0.04±0.01	C
Wosr+ Wfa hi w 50	0.05±0.02	C
Wosr hi n	0.00±0.00	D
Wosr hi w	0.00±0.00	D
Wosr lo w	0.00±0.00	D

The dry weight of legumes showed some significant variation across the treatments, with some treatments exhibiting much higher biomass than others, as indicated by the results in Table 12. The treatment WOSR with wfa lo w 50 had the highest mean dry weight of 0.21g, which was significantly higher than all other treatments. This implies that the interaction between WOSR and wfa lo w 50 may provide the best conditions for legume growth, which in turn leads to increased biomass.

On the other hand, WOSR with fs fa lo w (0.04g) and WOSR with fs fa hi (0.02g) had significantly lower dry weights, which means that frost-sensitive legume species (fs) were not efficient in terms of biomass production, especially when mixed with WOSR. This shows that there is a potential weakness in the use of frost-sensitive legume varieties in some growing conditions (frost) since they do not contribute much to the legume biomass. Notably, the frost-tolerant legume treatments WOSR+ wp (0.16g) and WOSR+ wfa (0.14g) also had higher biomass than the frost-sensitive treatments but were not as high as the WOSR with wfa lo w 50. This further supports the idea that frost-tolerant legumes could enhance legume biomass, although not as much as frost-tolerant varieties such as wfa.

In addition, the treatments with WOSR and row spacing including WOSR+ wp lo w 50 (0.01g) and WOSR with wp lo w 25 (0.04g) had very low legume biomass, especially in the wide row spacing treatments. This implies that row spacing could be used to regulate legume growth, whereby wider spacing could slow down biomass accumulation because of low competition and cover.

Taken together, it demonstrates that more consideration should be paid to the proper choice of the legume species (frost-tolerant vs. frost-sensitive), row width, and variety in order to enhance the legume biomass as a potential factor for improving nitrogen capture and general soil quality.

4.6. Dry weight of weed biomass:

The dry weight for all 13 weeds activity analysis of variance (ANOVA) revealed significantly significant differences ($p < 0.05$) between some treatments. Significant variability across the treatment groups is shown by the F-calculated value of 336.92, which highlights the effect of various treatments on dry weight in weeds.

Table 13: Analysis of variance for dry weight (g) in weeds

Source	DF	SS	MS	F	P
Replication	3	2.012	6.70		
Treatment	12	0.00394	3.283	336.92	0.0000*
Error	36	3.508	9.744		
Total	51	0.00398			

* = Significant (P≤0.05), ns= Non-significant

Table 14: Mean comparison test of various treatments for weed dry matter biomass. Treatments within the same homogenous group (sharing the same letter) are not significantly different.

Treatment	Mean (g/0.75 sqm) ± S.D	Homogenous groups
Wosr hi w	0.036±0.004	A
Wosr lo w	0.026±0.004	B
Wosr+ Wfa hi w 50	0.024±0.004	B
Wosr+ fs fa lo w	0.018±0.002	C
Wfa	0.015±0.003	D
Wosr with wfa lo w 50	0.015±0.002	DE
Wosr with fs fa hi	0.014±0.002	DE
Wosr with wfa lo w 25	0.012±0.002	EF
Wosr with wp lo w 25	0.010±0.002	FG
Wosr+ wp lo w 50	0.008±0.001	GH
Wosr hi n	0.007±0.001	H
Wosr+ Wp hi w 50	0.006±0.001	H
Wp	0.005±0.001	H

The mean comparison test for weed dry matter (g) biomass, as shown in Table 14, reveals few significant differences in the effectiveness of various treatments in terms of weed growth. The highest dry weight of weeds was observed in the treatment WOSR hi w (0.036g), which belongs to the top homogeneous group (A), indicating that this treatment allowed for greater weed growth compared to others. In contrast, the lowest dry weight of weeds was found in Wp (0.005g), which was part of the H group, reflecting very little weed biomass in this treatment.

The WOSR hi w treatment, with the highest weed dry weight, was followed closely by WOSR lo w (0.026g) and WOSR+ wfa hi w 50 (0.024g), which also exhibited significant

weed biomass, placing them in the B group. These treatments seem to allow for more favorable conditions for weed growth, potentially due to factors like row spacing or the specific legume combination used, which may not effectively suppress weed growth.
On the other hand, treatments involving WOSR with frost-sensitive or frost-tolerant legumes, such as WOSR with fs fa lo w (0.018g) and WOSR with wfa lo w 50 (0.015g), showed lower weed biomass and were categorized in the C and D groups. These results suggest that the inclusion of frost-sensitive or frost-tolerant legumes may reduce weed growth compared to treatments with less effective legume species or combinations.

Further, the WOSR+ wp lo w 50 (0.008g) and WOSR with wp lo w 25 (0.010g) treatments, as well as other treatments in the lower groups, showed even less weed biomass, suggesting that row spacing and possibly the choice of legumes combined with inter-row hoeing played a role in reducing weed competition. WOSR hi n (0.007g) and WOSR+ wp hi w 50 (0.006g) also demonstrated low weed growth, reinforcing the idea that certain legume varieties or configuration are more effective in limiting weed establishment.

4.7. Dry weight of WOSR

The analysis of variance (ANOVA) for WOSR dry weight demonstrated some significant variations among 11 treatments, suggesting that different management approaches substantially influence biomass production.

Table 15: Analysis of variance for WOSR dry weight under different treatments

Source	DF	SS	MS	F	P
Replication	3	0.00012	0.00004		
Treatment	12	0.02472	0.00206	72.04	0.0000*
Error	36	0.00103	0.00003		
Total	51	0.02587			

* = Significant (P≤0.05), ns= Non-significant

Table 16: Mean comparison test of various treatment for dry weight (g) in WOSR. Treatments within the same homogenous group (sharing the same letter) are not significantly different.

Treatment	Mean (g/0.75 sqm) ± S.D	Homogenous groups
Wosr hi n	0.091±0.004	A
Wosr+ wp lo w 50	0.032±0.003	B
Wosr with wfa lo w 25	0.025±0.003	BC
Wosr hi w	0.024±0.003	BCD
Wosr lo w	0.017±0.002	CD
Wosr with wfa lo w 50	0.016±0.002	CD
Wosr with wp lo w 25	0.016±0.002	CD
Wosr+ Wp hi w 50	0.015±0.002	CD
Wosr with fs fa hi	0.014±0.001	CD
Wosr+ fs fa lo w	0.012±0.001	CDE
Wosr + Wfa hi w 50	0.011±0.001	DE
Wfa	0.000±0.000	E
Wp	0.000±0.000	E

The WOSR high nitrogen treatment, characterized by elevated fertilizer application and narrow row spacing, demonstrated the greatest dry weight (0.09), indicating that both optimal nutrient availability and diminished resource competition facilitated increased biomass. Narrow row spacing facilitates more effective distribution of light and nutrients, while elevated fertilizer inputs guarantee ample necessary nutrients for robust development. Conversely, intercropping treatments, specifically WOSR with wp lo w 50 and WOSR with wfa lo w 25, exhibited diminished dry weights (0.032 g and 0.025 g, respectively), as interspecific competition for resources, notably nitrogen, constrained WOSR growth. Increased row spacing and diminished fertilizer application further limited biomass production, underscoring the trade-offs between the advantages of intercropping and the productivity of winter oilseed rape (WOSR).

Intercropping treatments involving frost-sensitive legumes, such as WOSR + fs fa lo w (0.012 g) and WOSR with fs fa hi (0.014 g), resulted in even lower dry weights, indicating that these legumes' reduced nitrogen fixation capabilities and higher competitiveness suppressed WOSR growth. Moreover, minimal fertilizer application in these treatments significantly restricted the nutrients accessible to WOSR, intensifying the adverse effects on biomass accumulation. Exclusive legume treatments such as wfa and wp yielded no quantifiable WOSR dry weight, as these plots were solely allocated for legume cultivation, excluding WOSR. The data indicate that elevated fertilizer applications and tight row spacing substantially increase WOSR biomass, but intercropping and decreased input systems provide ecological advantages at the expense of marginally diminished crop yields. The findings emphasize the significance of choosing suitable legume species and modifying row spacing to enhance biomass output in intercropping systems.

4.8. Total Nitrogen level (%) in WOSR biomass

The analysis of variance (ANOVA) for the Nitrogen level (%) in the WOSR for 11 treatments are shown in table 17. It was indicated that all treatments were non significantly distinctive from one another. LSD test indicated that those treatments have the same alphabetic letters means that these treatments have the same results for Nitrogen level (%) in the WOSR and showed non-significant results compared to each other. All treatments had non-significant differences among them.

Table 17. Analysis of variance for Nitrogen level (%) in the WOSR

Source	DF	SS	MS	F	P
Replication	3	3.5440	1.8134		
Treatment	10	7.0752	0.70752	0.83	0.6023ns
Error	30	25.5190	0.85063		
Total	43	36.1383			

* = Significant (P≤0.05), ns= Non-significant

Table 18. Response of treatments against nitrogen level in the WOSR

Treatment	Mean (%) ± S.D	Homogenous group
WOSR+ wp hi w 50	5.97±0.07	A
WOSR+ fs fa lo w	5.49±0.04	A
WOSR hi w	5.31±0.06	A
WOSR+ wfa lo w 50	5.30±0.08	A
WOSR+ wfa hi w 50	5.30±0.11	A
WOSR+ wfa lo w 25	5.26±0.06	A
WOSR+ fs fa hi w	5.22±0.12	A
WOSR lo w	5.04±0.06	A
WOSR+ wp lo w 25	4.74±0.09	A
WOSR+ wp lo w 25	4.61±0.06	A
WOSR hi n	4.51±0.06	A

The nitrogen content in WOSR (Winter Oilseed Rape) was not significantly not influenced by the treatment factors, as indicated by the data in Table 18. The highest nitrogen levels were found in the treatment WOSR+ wp hi w 50 (5.97 %), which had the highest nitrogen concentration among all treatments, followed closely by WOSR+ fs fa lo w (5.49 %). Both treatments belong to the same homogeneous group (A), indicating that their nitrogen levels were not significantly different from one another.

4.9. Total Nitrogen level (%) in legume biomass

The analysis of variance (ANOVA) for the nitrogen level (%) in the legumes for 10 treatments are shown in table 19. It indicates that all treatments were highly non-significantly distinctive from one another.

Table 19. Analysis of variance for nitrogen level in the legumes

Source	DF	SS	MS	F	P
Replication	3	2.2983	0.76609		
Treatment	9	1.7137	0.19041	0.31	0.9647ns
Error	27	16.5586	0.61328		
Total	39	20.5706			

* = Significant ($P \leq 0.05$), ns= Non-significant

Table 20. Response of treatments against nitrogen level (%) in the legumes

Treatment	Mean (%) ± S.D	Homogenous groups
WOSR+ wp lo w 50	3.08±0.05	A
WOSR+ fs fa hi w	3.05±0.05	A
WOSR+ wfa lo w 25	2.69±0.05	A
Wfa	2.65±0.05	A
WOSR+ wp lo w 25 del	2.64±0.04	A
WOSR+ fs fa lo w	2.59±0.04	A
WOSR+ wfa lo w 50	2.58±0.03	A
WOSR+ wp hi w 50	2.54±0.04	A
Wp	2.49±0.04	A
WOSR+ wfa hi w 50	2.45±0.05	A

According to table 20, The nitrogen levels across the treatments are non significant. Highest nitrogen % was found in WOSR intercropped with winter pea with low N inputs, wide spacing at 50 % plant density while lowest nitrogen % was found in WOSR intercropped with high N inputs, wide spacing at 50 % plant density. Both belongs to homogenous group A.

4.10. Total Nitrogen level (%) in weed biomass

The analysis of variance (ANOVA) for the nitrogen level in the weeds for 13 treatments are shown in table 21. It was indicated that all treatments were highly non-significantly distinctive from one another.

Table 21 Analysis of variance for nitrogen level (%) in the weed biomass

Source	DF	SS	MS	F	P
Replication	3	11.2640	3.75467		
Treatment	12	14.9859	1.24883	1.27	0.2763ns
Error	36	35.3358	0.98155		
Total	51	61.5857			

* = Significant (P≤0.05), ns= Non-significant

Table 22. Response of treatments against nitrogen level (%) in the weed biomass

Treatment	Mean (%) ± S.D	Homogenous groups
WOSR+ wfa lo w 50	4.04±0.04	A
WOSR+ wfa lo w 25	4.01±0.04	A
Wp	3.77±0.05	A
WOSR+ wp hi w 50	3.44±0.04	A
WOSR+ fs fa lo w	3.32±0.03	A
WOSR+ fs fa hi w	3.21±0.03	A
WOSR+ wp lo w 25	3.09±0.04	A
WOSR+ wp lo w 50	3.04±0.06	A
WOSR hi w	3.02±0.04	A
WOSR hi n	2.94±0.04	A
Wfa	2.79±0.05	A
WOSR+ wfa hi w 50	2.60±0.04	A
WOSR lo w	2.04±0.05	A

According to Table 22, the maximum nitrogen concentrations in weeds were recorded in WOSR + wfa lo w 50 (4.04%) and WOSR + wfa lo w 25 (4.01%). Reduced nitrogen concentrations in weeds were noted in treatments such as WOSR lo w (2.04%) and WOSR + wfa high 50 (2.60%). All these treatments belong to homogeneous group A as the results were non-significant.

4.11. Correlation analysis of CCI and N concentration of WOSR plants

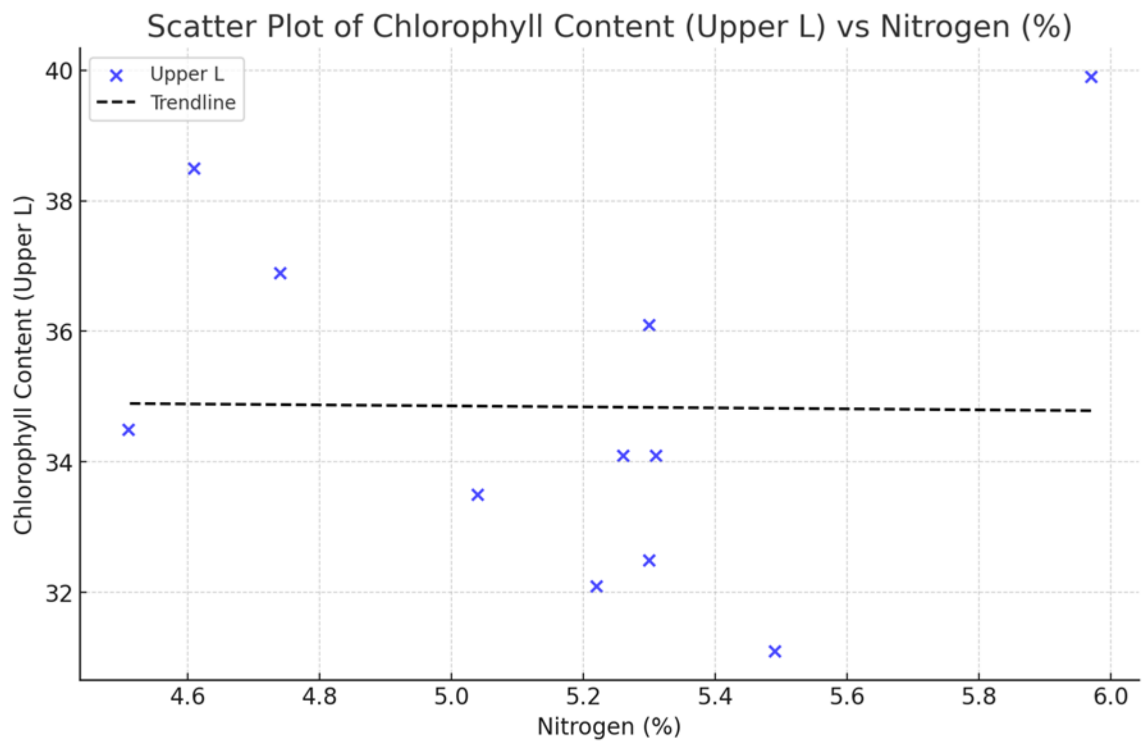

Figure 2. Scatter plot of N% and CCI of Upper leaves

The Spearman's rank correlation analysis of the relationship between nitrogen concentration (N%) and the chlorophyll content in the upper leaves was performed. It is observed very weak negatively correlated ($\rho=-0.182$) and weak P value ($0.59 > 0.05$) showing that there is no significant relationship between two variables, N concentration slightly increase, and slightly decrease chlorophyll, which is not strongly associated. A scatter plot (Figure 2) with a fitted trendline provides further evidence to this finding as we do not see any strong monotonic relationship present between two variable.

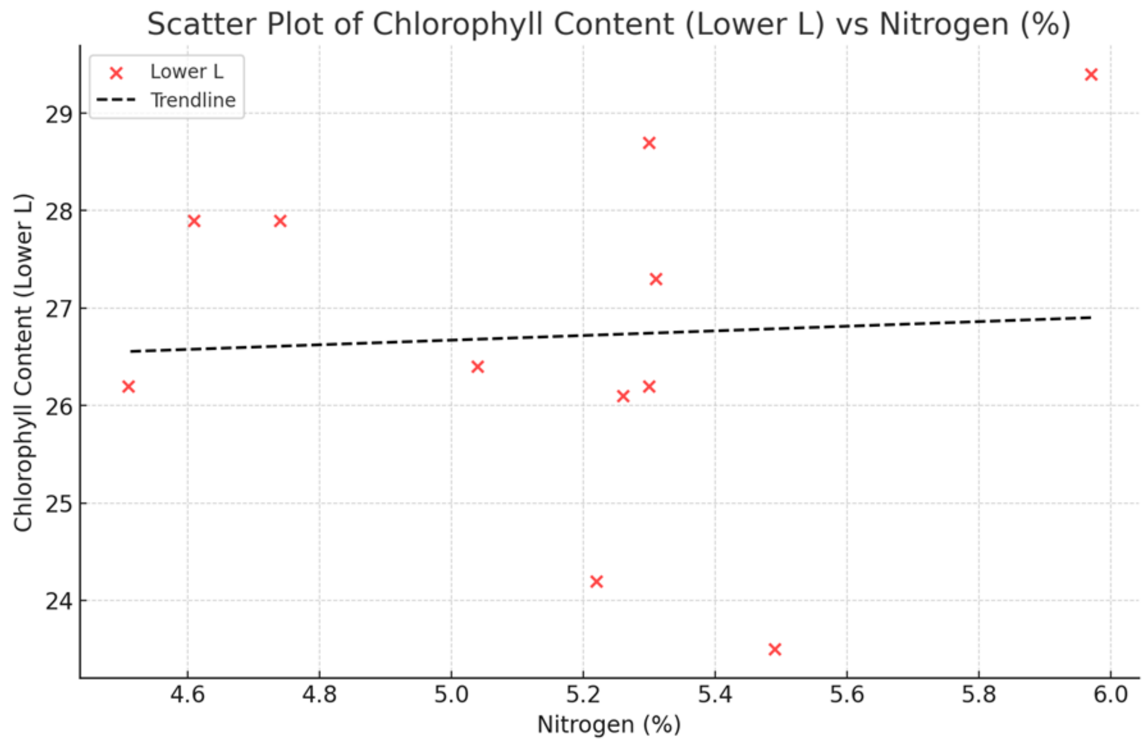

Figure 3. Scatter plot of N% and CCI of Lower leaves

Spearman's rank correlation was also used to examine the connection between lower leaves' nitrogen concentration (N%) and chlorophyll content (CCI). The results showed that there was no statistically significant association between the two variables, despite a very weak positive correlation ($\rho=0.052$) and P value ($0.877 > 0.05$), which is different from upper leaves. Since there is no discernible trend and the data points are widely scattered, the scatter plot (Figure 3) further demonstrates the poor connection. To illustrate the overall direction of the data, a trendline was drawn; it indicated a modestly positive trend.

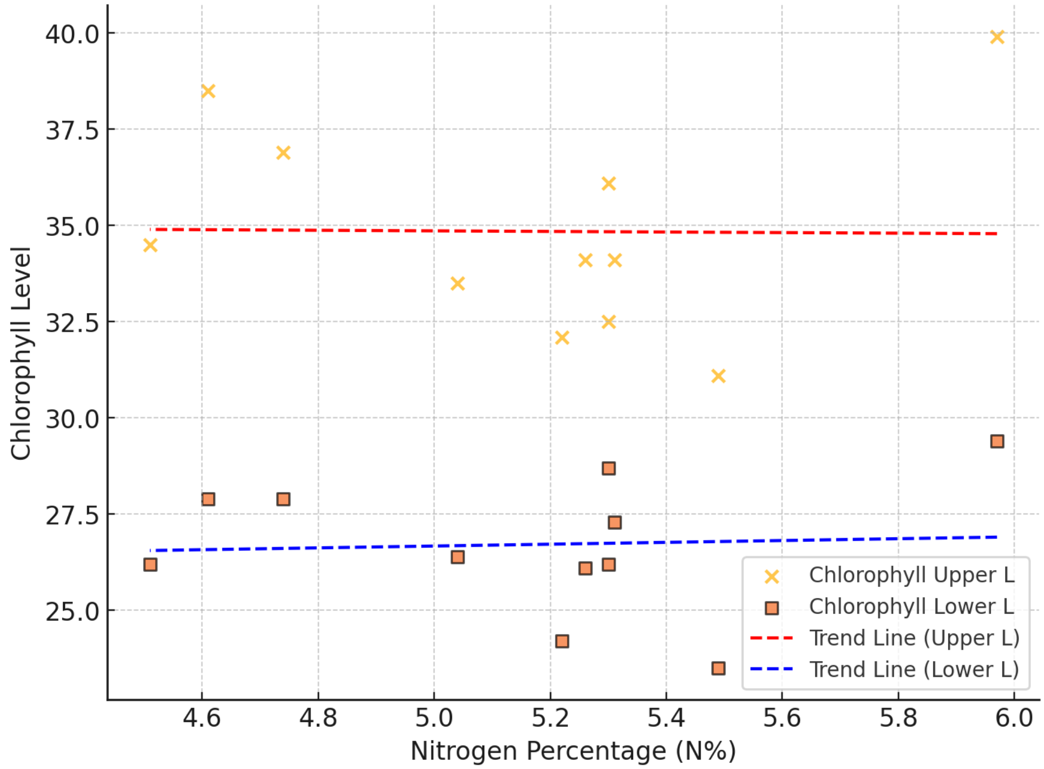

Figure 4. Combined Scatter Plot of N% and Chlorophyll content of upper and lower leaves.

DISCUSSION

5.1. Chlorophyll content in Upper leaves

The WOSR intercropped with over-wintering pea, reduced inputs, broad row spacing, and 25% sowing density of over-wintering pea seeded six weeks after WOSR treatment demonstrated the maximum chlorophyll content. The lowest chlorophyll levels were seen in *WOSR intercropped with frost-sensitive faba bean, characterized by lower inputs, wide row spacing, and simultaneous sowing with WOSR.* The results indicate that specific intercropping treatments markedly boost photosynthesis, presumably due to enhanced light interception and nutrient absorption.

The LSD test supported these conclusions by revealing distinct clusters of all treatments. High levels of amine chlorophyll especially in the particular treatments indicate high plant health and potential increased yield. The right spacing and efficient utilization of available light and nutrients in treatments such as WOSR intercropped with over-wintering pea promoted growth and thus increased chlorophyll content. This concurs with Chen et al. (2017) who proved that legume cereal intercropping improves chlorophyll status by promoting light interception and nutrient utilization. Zhang et al. (2022) also showed that the microenvironments created by intercropping systems improve the photosynthetic rate.

The high chlorophyll content in the *WOSR intercropped with over-wintering pea, low inputs, wide row spacing, and 25% sowing density of over-wintering pea sown ten days after WOSR* treatment can be attributed to the combined effect of wide-row spacing and delayed sowing of pea. The delayed planting allowed WOSR to establish a competitive canopy and achieve a desirable plant growth stage thereby minimizing competition for resources. These results underscore the importance of timing and the choice of companion crop in enhancing chlorophyll synthesis. Furthermore, the findings vindicate the claims made by Szumigalski and Van Acker (2005) according to whom strategic intercropping increases chlorophyll levels by boosting photosynthesis productivity.

Treatments involving frost-tolerant legumes were generally higher than those involving frost-sensitive legumes. Perennial legumes would be beneficial for increasing nutrient cycling and light interception, but annual frost-sensitive legumes would be disadvantageous to the WOSR growth due to reduced ability of nitrogen fixation and increased competition under stress conditions. This distinction underscores the significance of legume variety in attaining enhanced chlorophyll levels and crop efficacy within intercropping systems.

5.2. Chlorophyll content in lower leaves

The highest chlorophyll concentrations were recorded in *WOSR intercropped with over-wintering pea, characterized by decreased inputs, broad row spacing, and a 25% sowing density of over-wintering pea seeded six weeks subsequent to WOSR.* In contrast, the minimal levels were observed *in WOSR intercropped with frost-sensitive clover, characterized by high input levels, tight row spacing, and frost-sensitive clover sowed concurrently with WOSR.* Consistently elevated chlorophyll concentrations in both upper and lower leaves signify that certain treatments ensure homogeneous growth conditions essential for optimal photosynthetic efficiency and agricultural yield.

Studying chlorophyll content in different parts of the leaf on plants shows that intercropping enhances light penetration and nutrient distribution, which enhances plant health. Treatments involving peas showed a higher chlorophyll content in the lower leaves, which makes them suitable for intercropping systems. However, the treatments, where intercropped faba beans were applied, sometimes caused a decrease in chlorophyll content, probably due to shading or nutrients competition.

The results are in agreement with Lithourgidis et al. (2011) who noted that intercropping systems improve the health of individual plant parts through efficient resource allocation. The results also support the findings of Szumigalski and Van Acker (2005) that strategic intercropping can increase the total chlorophyll content in the plant.

Notably, *WOSR intercropped with over-wintering peas, reduced inputs, wide row spacing, and 25% sowing density of over-wintering peas sown six weeks after WOSR showed the highest chlorophyll concentrations in both the upper and lower leaves*. Explicitly, the application of broad rows, delayed pea planting, and reduced planting density may avoid the shadow effect and complementary contest for resources, thus promoting photosynthetic capacity. In addition, the delay of sowing the legume provided WOSR plants to grow a competitive canopy in terms of resource capture and photosynthesis. These results suggest that companion crop density and planting times should be well controlled in intercropping systems.

The low efficiency of treatments including frost-sensitive legumes such as *WOSR intercropped with frost-sensitive clover, high input levels, narrow row spacing*, and *WOSR intercropped with frost-sensitive faba bean, low inputs, wide row spacing* shows that frost sensitivity reduces chlorophyll formation. These legumes may restrict nutrient accessibility and light infiltration, diminishing overall plant vitality and yield.

5.3. Comparative Examination of Chlorophyll Concentrations in Upper and lower leaves

The *WOSR intercropped with over-wintering pea, reduced inputs, wide row spacing, 25% sowing density of wp sown six weeks after WOSR* treatment consistently demonstrated the highest chlorophyll concentrations in both higher (41.1 CCI) and lower (31.2 CCI) leaves. This underscores the effectiveness of diminished sowing density, broad row spacing, and postponed sowing in facilitating equal chlorophyll distribution across the plant canopy. The decreased pea density lessened inter-plant competition, facilitating fair resource distribution and alleviating shadowing impacts, especially on the lower leaves.

The lowest chlorophyll levels were seen in treatments involving frost-sensitive legumes, specifically *WOSR intercropped with frost-sensitive faba bean, reduced inputs, wide row spacing, fs fa sown on same date as WOSR* and *WOSR intercropped with frost-sensitive clover (fs cl), high input levels, narrow row spacing, fs cl sown on same date as WOSR*. The inadequate efficacy of these treatments highlights the adverse impacts of frost sensitivity and insufficient nitrogen fixation on chlorophyll synthesis.

Intermediate treatments, specifically *WOSR intercropped with over-wintering pea, reduced inputs, wide row spacing, 50% sowing density of wp sown six weeks after WOSR* and The *WOSR intercropped with over-wintering pea, high inputs, wide row spacing, 50 sowing density*, demonstrated comparatively elevated chlorophyll concentrations in both upper and lower leaves; however, they did not surpass the performance of *WOSR intercropped with over-wintering pea, reduced inputs, wide row spacing, 25% sowing density of wp sown six weeks after WOSR*, this indicates that although wide-row spacing and nitrogen administration are advantageous, decreasing sowing density and postponing legume planting further enhance chlorophyll production. These findings underscore the necessity of equilibrating resource competition and light accessibility to attain maximum photosynthetic performance.

The disparity in chlorophyll concentrations between top and lower leaves was more evident in lower-performing treatments, signifying uneven resource allocation and inadequate growth conditions. Legumes that are frost resistant such as those used in *WOSR intercropped with over-wintering pea, low inputs, wide row spacing, 25% sowing density of wp sown six weeks after WOSR*, enhanced dispersion of chlorophyll and therefore their importance in enhancing plant health cannot be overemphasized.

These results highlight the interdependence of companion crop choice, planting density, and time in intercropping systems. Enhancing these parameters can markedly improve chlorophyll synthesis, photosynthetic efficacy, and agricultural yield. The results provide essential information for formulating successful intercropping methods to optimize plant health and production potential.

5.4. Weed cover Percentage

Significant variations were observed in weed coverage among the treatments. *The treatment WOSR intercropped with frost-sensitive clover, reduced inputs, narrow row spacing, fs cl sown on same date as WOSR* had the highest weed presence, while the lowest weed presence was in *WOSR intercropped with over-wintering pea, reduced inputs, wide row spacing, 25% sowing density of wp sown six weeks after WOSR with inter-row hoeing* . Effective weed suppression is critical for reducing competition for resources. The scarcity of weeds in treatments such as *WOSR intercropped with over-wintering pea, reduced* *inputs, wide row spacing, 25% sowing density of wp sown six weeks after WOSR* signifies a worthwhile crop competition which helps in not only the optimal resource use but also reducing herbicide requirements.

Intercropping has been documented to control weeds. According to Lithourgidis et al. (2011), intercropping can lead to reduced weed biomass, i.e., due to intensified competition from the main crop. Similarly, Szumigalski and Van Acker (2005) stated that intercropping systems enhanced weed competitiveness resulting into their stunted growth. Such tendencies are corroborated by our findings, where some intercropping strategies especially *WOSR intercropped with over-wintering pea, reduced inputs, wide row spacing, 25% sowing density of wp sown six weeks after WOSR with inter-row hoeing* proved to be very effective in containing weed presence. In other words, lower numbers of weeds being found among treatments like *WOSR intercropped with over-wintering pea, reduced inputs, wide row spacing, 25% sowing density of wp sown six weeks after WOSR* reflect a strong competitive ability of this crop that has advantages for saving resources and decreasing herbicides application.

Intercropping is known to have the potentiality to reduce weed pressure on crops. Moreover, according to Lithourgidis et al. (2011), it can cause substantial decrease in weed biomass due to increased intra-specific competition by crops grown together as compared with sole cropping systems. Similarly, intercropping systems were mentioned as more competitive environments for weeds thus showing their retardation (Szumigalski and Van Acker 2005). In this context, some intercropping systems— particularly *WOSR intercropped with over-wintering pea, reduced inputs, wide row spacing, 25% sowing density of wp sown six weeks after WOSR* with inter-row hoeingwere highly efficient at minimizing the appearance of weed species quite similar from those pointed out above. Thus, less number of weeds appearing on treatments like those based on *WOSR+ wp lo w 25* reflect high plant competitiveness which is beneficial for resource conservation or cutting down on herbicides use.

5.5. Dry Weight of Legumes, weeds and WOSR

When the treatment of Winter Oilseed Rape (WOSR)is intercropped with over-wintering faba bean (wfa) low input and wide spacing at 50% plant density, it exhibited the highest mean dry weight of 0.21g, greatly surpassing all other treatments. This indicates that the particular combination of *WOSR with diminished inputs, broad row spacing, and a 50% seeding density of over-wintering faba bean sown ten days post-WOSR* creates an ideal environment for legume growth, enhancing biomass accumulation. The increased biomass production observed in this treatment highlights the importance of careful attention to the intercropping systems to promote legume vigor and biomass accumulation.

The findings are in concordance with literature suggesting that efficient intercropping systems enhance nutrient cycling and resource use hence increasing biomass accumulation. According to Agegnehu et al (2014), advanced studies showed that legumes intercropped with cereals boost nitrogen fixation and nutritional balance and surge biomass. In line with these observations, the increased dry weight observed in *WOSR intercropped with over-wintering faba bean, combined with decreased inputs, wide row spacing, and 50% sowing density of over-wintering faba bean sown ten days after WOSR*, also shows the possibility of intercropping to enhance the growth and yield of legumes.

Treatments with frost sensitive legumes such as *WOR along with frost sensitive faba beans* had significantly low dry weight (0.02). This implies that frost sensitive legume species (fs) have lower efficiency in biomass production especially when grown in association with winter oilseed rape (WOSR). The reduced biomasses in these treatments might be attributed due to enhanced sensitivity of these legumes to frost, which presumably inhibits their growth and nitrogen-fixing capacity under the given environmental conditions. This partly points to the limitations of using frost-sensitive legume varieties when specific growth conditions are applied, under which they may not well support biomass yield as the frost-tolerant species do.

Treatments utilizing frost-tolerant legume species, such as *Winter Oilseed Rape combined with over-wintering pea (wp) (0.16g)* and with *over-wintering faba bean (wfa) (0.14g)*, demonstrated superior biomass accumulation compared to their frost-sensitive equivalents, yet did not attain the biomass levels of *WOSR with over-wintering faba bean low inputs, wide spacing at 50% seedling density*. This highlights the advantages of employing frost-resistant legume species, which exhibit greater resilience to environmental stress and can maintain biomass output throughout a broader spectrum of circumstances. However, while frost-tolerant legumes yield more biomass than frost-sensitive ones, their yield may not reach the level of species like over-wintering faba bean.

The effect of row spacing on legume biomass yield was also evident in the treatments where WOSR was planted at varying row spacing. *Winter Oilseed Rape + over-wintering pea low inputs, wide spacing at 50% (0.01g) and Winter Oilseed Rape + over-wintering pea low input, wide spacing at 25% (0.04g) had very low legume biomass, particularly in the wide-row spacing.* This was also apparent in the treatments with WOSR at different row spacings. The findings suggest that row spacing is important for legume growth, but larger spacing may limit biomass production. Reduced competition at wider row spacings may result in poor ground cover and reduced canopy which in turn affects the growth and biomass production of the legumes. This finding is in line with other studies that suggest that the best row spacing is critical in determining the efficient use of resources and light interception and nutrient supply to the legume crops.

The findings of this research will underscore the importance of identifying appropriate legume species - frost tolerant or frost sensitive - to be employed in intercropping systems. Furthermore, proper regulation of row spacing and seeding density is key to enhancing the legume biomass and turned out to be significant for increasing nitrogen fixation and soil health. The high biomass production obtained in treatments such as *WOSR intercropped with over-wintering faba bean, low inputs, and wide row spacing* demonstrate the importance of proper intercropping systems in enhancing legume growth and increasing agricultural yield. Furthermore, these findings are in agreement with Agegnehu et al. (2014) who pointed out that intercropping legumes with cereals significantly increase biomass yield and resource use efficiency.

The change in weed dry weight was observed throughout treatments, *the maximum weed biomass was recorded in the WOSR sole crop treatment having high input density and wide row spacing of 50 cm*. This treatment enhanced weed growth by reducing competition from the crop as a result of the treatment. However, the lowest weed biomass was recorded in the *WOSR intercropped with over-wintering pea (wp) with low inputs, broad row spacing, and 50% sowing density of wp sown ten days after WOSR with inter-row hoeing*, which indicated good weed control.

The diminished weed biomass in certain treatments indicates successful weed management, crucial for minimizing competition and enhancing crop yield. Treatments such as *WOSR intercropped with over-wintering pea* exhibited the efficacy of intercropping systems in weed management, corroborating the observations of Rao and Mathuva (2000), who indicated that heightened competition among species in intercropping systems inhibits weed proliferation. The mean comparison test for weed dry matter biomass (Table 12) indicated significant differences among the treatments.

Treatments involving WOSR combined with *over-wintering pea low input and wide spacing at 50% seeding density (0.008g) and WOSR with over-wintering pea low input and wide spacing at 25% seeding density (0.010g) with inter-row hoeing* exhibited reduced weed biomass, hence supporting the notion that particular legume cultivars and row spacing effectively mitigate weed competition. Intercropping with either frost-tolerant or frost-sensitive legumes is essential for controlling weed biomass. Choosing suitable intercropping companions, planting densities, and row configurations can markedly diminish weed proliferation, fostering cleaner fields and enhanced crop yield. These findings are in agreement with Rao and Mathuva (2000) who have established the effectiveness of intercropping.

Higher dry weight at this growth stage in winter oilseed rape is an indication of sound plant growth and biomass production, which are important for yield enhancement. The WOSR sole crop treatment had the highest dry weight as supported by previous research that showed that high input treatments such as full nitrogen fertilization and pesticide application increase biomass yield (Bedoussac et al. 2015). Other treatments such as *WOSR intercropped with an over-wintering pea at low sowing density (WOSR + wp lo w 50)* and *WOSR with over-wintering faba bean at low sowing density (WOSR with wfa lo w 25)* had significantly lower dry weights of 0.03g and 0.02g respectively.

These results imply that specific treatments for WOSR biomass production are required and that high inputs and close row spacing are important for maximum dry weight. Although intercropping systems can increase biomass through complementary species, the WOSR lone crop treatment, when managed appropriately, is essential for optimizing production in this study.

5.6. Nitrogen Levels in WOSR, legumes and weeds

Although there were no significant differences in nitrogen levels among the WOSR, these observations are however vague and based on the numerical values of the treatments. The treatment that had highest nitrogen level was *WOSR intercropped with over-wintering pea (wp), high input levels, wide row spacing, 50% sowing density of wp sown ten days after WOSR*, followed by *WOSR intercropped with frost-sensitive faba bean, reduced inputs, wide row spacing, fs fa sown on same date as WOSR and WOSR sole crop, high input levels wide row spacing.*

The presence of stable nitrogen levels in the treatments suggests good nitrogen management which is typical of legumes capable of fixing nitrogen. Some specific treatments have higher levels of nitrogen, thus better growth and more production. This has been well-documented about legumes enhancing nitrogen availability. For example, Rao and Mathuva (2000) found out that using legume intercrops with cereals increase soil's nitrate content that is beneficial to associated crops. These results also confirm findings by others who observed higher N levels in *WOSR intercropped with over-wintering pea (wp), high input levels, wide row spacing, 50% sowing density of wp sown ten days after WOSR* indicating effective intercropping increased N availability leading to improved crop performance.

In legumes, there was no significant difference among treatments as it was indicated in ANOVA for nitrogen levels and again the following debate is based on numerical values of treatments. The highest nitrogen content was recorded in *WOSR intercropped with over-wintering pea, low input, large row spacing, and 50% sowing density of wp sown ten days after WOSR*. Therefore, legumes have the potential for making WOSR's fertility as well as improving the performance of crops through the fixation of nitrogen. This high state of nitrogen content in legumes is an indication of the successful uptake of the nutrient and its benefits to associated crops. Different researchers have stressed the ability of legumes to affect nitrogen fixation together with enhancing other crops grown in the same field. This has been explained by Agegnehu et al. (2014) who indicated that it was possible to improve soil nitrogen fixing and fertility through the adoption of practices such as legume/cereal intercropping. These results are in agreement with other studies that revealed that some of the treatments contained significantly higher N than others, and therefore, the intercrops systems are effective in increasing the availability of N.

The weed treatments did not differ in nitrogen concentrations as they possess statistical non-significant results. However, the observations are based on numerical values of the treatments as previously stated. The highest levels were observed in *WOSR intercropped with over-wintering faba bean, reduced inputs, wide row spacing, 50% sowing density of wfa sown ten days after WOSR*, and with the lowest levels in *WOSR sole crop, reduced inputs, wide row spacing*. This is why nitrogen levels in weeds are best monitored to understand nutrient competition among the weeds. Controlling nitrogen in weeds can minimize their competitive advantage, thereby improving crop nutrient availability. Nutrient dynamics within intercropping systems are complex and demand careful study for effective management practices. Szumigalski and Van Acker (2005) found that intercropping can influence nutrient distribution patterns, which significantly impact weed growth rates. These findings align with studies on legume-based intercropping systems, where nitrogen-fixing species, such as clover or cowpea, tend to enhance soil nitrogen levels, reducing nitrogen availability for weeds in some scenarios. However, other studies report contrasting results, showing increased weed competition in systems where nitrogen levels exceed crop requirements.

5.7. Correlation analysis of CCI and N concentration of WOSR plants

Usually, a positive correlation exists between leaf nitrogen concentration and chlorophyll content due to the fact that nitrogen is a basic part of chlorophyll molecules and necessary for photosynthesis. However, the weak negative correlation observed in upper leaves might reflect a combination of multiple factors. Since leaves in the upper canopy are exposed to higher light intensities, light saturation may be the cause. Under these circumstances, more nitrogen might not improve photosynthetic rates or chlorophyll synthesis any more, which could lead to a plateau or even a drop in chlorophyll concentration. In a study by Padilla et al. (2018), this phenomenon was noted: at high nitrogen levels, the amount of chlorophyll in leaves approaches a plateau, suggesting that more nitrogen does not raise chlorophyll concentrations after a certain point.

However, nutritional imbalances can also play a significant role in the issue because too much nitrogen application can upset the equilibrium of other key elements, including potassium and phosphorus, which are also necessary for the synthesis of chlorophyll and the general health of plants. Since plants need a balanced nutrient supply for optimal growth, this imbalance may have a negative impact on the amount of chlorophyll (Yuan et al. 2023).

On the other hand, In the lower canopy, the leaves receive less light exposure and frequently adjust by raising their chlorophyll concentration to enhance light collection, it is possible to claim that the modest positive association between N% and chlorophyll content in lower leaves is due to their adaptation to shadowed situations. They can continue to photosynthesize efficiently in low light because of this adaption. According to studies, lower canopy foliage has a substantially greater mass-based chlorophyll concentration than upper canopy leaves. This suggests that mechanisms other than nitrogen concentration may be more important in causing chlorophyll accumulation in lower leaves (Gardner et al. 2022).

Since plants preferentially distribute nitrogen to upper, more light-exposed leaves in order to maximize photosynthesis, nitrogen redistribution may also potentially be a contributing factor. In order to maintain their photosynthetic activity, lower leaves may get less nitrogen but make up for it by retaining or even raising the content of chlorophyll. Despite receiving less nitrogen, this tactic guarantees that lower leaves continue to contribute to the plant's overall energy production (Linders et al. 2024).

These conclusions are supported by Evans & Clarke's (2019) research, which demonstrates that chlorophyll concentration is influenced by variables other than nitrogen availability, such as photosynthetic efficiency, nutrient partitioning, and environmental circumstances. Although the data demonstrates that nitrogen is still necessary, its effects on chlorophyll concentration may be obscured by other physiological and environmental factors, potentially leading to statistically insignificant results across treatments.

However, according to our results, the connection between N% and CCI in the upper and lower leaves, respectively, is either negative or very weakly positive. Similar observation have been made in past as according to research by Johansson (2023), intercropping with legumes increased the N content in WOSR in one year of the trial, but this effect was not observed in the following year. Because of this, drawing firm conclusions from the data we gathered for our study is challenging. Additionally, research by Bedoussac et al. (2015) found that in intercropped systems, the main crop's N content increased but the legumes' aerial dry weight considerably decreased due to intense competition with the main crop. This opens the door for more research inquiries and studies and also shows the different ways in which the actors are associated with one another during the intercropping system.

5.8. WOSR Sole crop performance

The results of single-crop treatments in this study revealed significant variations in the important factors, which showed the effects of management practices on growth processes and nutrient utilization. The higher weed biomass observed in the WOSR hi w treatment, even though the species has a natural competitive ability, points to a limitation of high-input systems combined with wide row spacing. This layout might unintentionally promote the development of weeds since these factors mean increased access to the resources that are necessary for plants' growth – light, space, and nutrients reduce a crop's competitive ability.

On the other hand, dry weight measurement showed that WOSR hi n was the most productive treatment, implying that narrow row spacing and high input levels boosted WOSR biomass production. This result provides credence to the idea that increased planting density can increase competition for resources between plants and suppress weed growth. However, the nutrient content metrics of WOSR hi n were not as advantageous as its dry weight, which is beneficial. It was consistently lower in nitrogen and carbon, suggesting that the efficiency of nutrient uptake was limited or that nutrient partitioning was suboptimal under these high-input regimes.

CONCLUSION

The present research provides valuable information on the role of intercropping systems involving WOSR and legumes with regard to increase biomass, weed competitiveness, chlorophyll and nitrogen content. The findings suggest that growing winter oilseed rape with frost-tolerant legumes like over-wintering pea and faba bean significantly enhances crop biomass, nitrogen and chlorophyll content and effectively suppresses weeds in early spring. These results reveal the potential of intercropping systems in improving resource use and reducing competition between species, thus improving crop yield and ecosystem performance.

The analysis of nitrogen (N) content in plant biomass showed non-significant distinctions between intercropping and monoculture practices. Intercropped treatments showed higher nitrogen content (based on the numerical values), which supports the use of legumes in nitrogen accumulation and improvement of soil fertility. Compared to intercropped treatments, sole WOSR systems showed comparatively low concentrations of nitrogen and chlorophyll, despite producing significant biomass under high input levels. Even while they generate more biomass, they could not possess the qualities of intercropping systems that helped achieve overall sustainability.

The complex interplay between chlorophyll content and nitrogen availability across several canopy layers. They emphasize how crucial it is to take into account light exposure, leaf position, and nutrient distribution when evaluating the variables affecting plants' accumulation of chlorophyll. More studies that take these factors into account will improve our knowledge of the dynamics of plant nutrients and help develop more efficient fertilization techniques.

Overall study shows that WOSR-legume intercropping is a sustainable agricultural practice that offers many benefits including improved nitrogen availability, enhanced photosynthesis, weed suppression and reduced the reliance on synthetic fertilizer application. To some extent, these systems provide a good foundation for addressing the challenges of modern agriculture by increasing efficiency in the use of resources, promoting nitrogen storage, and controlling weeds. More study is needed to know how intercropping influences the crop performance over the long term under different climatic and growing conditions, combine with N applications and mechanical weeding with different row distances, to define further the particulars of the intercropping systems, and to compare their performance in different circumstances. These initiatives will improve the development of sound and productive agroecosystems that will meet the food security needs of the world.

Popular Science Summary

Adopting sustainable agricultural techniques has become more and more important as global agriculture faces growing problems. The purpose of this project is to determine if intercropping winter oilseed rape (WOSR) with legumes, including faba beans and overwintering peas, may increase crop output, increase resource efficiency, and support environmental sustainability.

The study, which was carried out at the Swedish University of Agricultural Sciences' (SLU) Lönnstorp research station, examined 24 different WOSR-legume intercropping regimens. The results showed that the maximum chlorophyll content and the greatest reduction in weed density were obtained when WOSR was interplanted with overwintering peas utilizing low-input techniques, wide row spacing, and reduced sowing density. Likewise, the maximum nitrogen content and legume biomass were obtained via intercropping with faba beans, which improved nutrient availability.

When compared to WOSR cultivation alone, the study showed that intercropped systems had better nitrogen utilization efficiency. Farmers may lessen their reliance on synthetic fertilizers thanks to this advancement, which lowers expenses and lessens the effects on the environment.

In addition to increasing output, intercropping provides a natural way to control weeds, lowering the need for chemical pesticides and building a more robust agroecosystem. These advantages, which maximize resource use and improve biodiversity, are consistent with the more general objectives of sustainable agriculture.

In summary, WOSR-legume intercropping offers a viable way to strike a compromise between ecological care and agricultural output. To evaluate the long-term impacts on soil health and to optimize intercropping practices for diverse environmental circumstances, more research is required. Systems for producing food that are robust, effective, and ecologically conscious can greatly benefit from adopting such sustainable approaches.

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APPENDIX

Layout and distribution of treatments over four blocks are following:

Table 23. Replicated blocks description

	plot	Led	Treatment (led) abbreviation		plot	led	Treatment (led) abbreviation
Block I	1	P	WOSR+wp hi w 50	Block II	25	O	WOSR+wfa lo w 25 del
	2	V	wfa del		26	E	WOSR+fs cl lo n
	3	K	WOSR+wfa hi w 50		27	T	WOSR+wp lo w 25 del
	4	I	WOSR+fs fa lo w		28	L	WOSR+wfa lo w 50
	5	R	WOSR+wp lo w 50 del		29	U	Wfa
	6	T	WOSR+wp lo w 25 del		30	I	WOSR+fs fa lo w
	7	A	WOSR hi n		31	Q	WOSR+wp lo w 50
	8	U	Wfa		32	N	WOSR+wfa lo w 25
	9	X	wp del		33	B	WOSR hi w
	10	G	WOSR+fs cl lo w del		34	S	WOSR+wp lo w 25
	11	F	WOSR+fs cl lo w		35	K	WOSR+wfa hi w 50
	12	B	WOSR hi w		36	W	Wp
	13	E	WOSR+fs cl lo n		37	F	WOSR+fs cl lo w
	14	Q	WOSR+wp lo w 50		38	V	wfa del
	15	C	WOSR lo w		39	D	WOSR+fs cl hi n
	16	W	Wp		40	M	WOSR+wfa lo w 50 del
	17	D	WOSR+fs cl hi n		41	J	WOSR+fs fa lo w del
	18	O	WOSR+wfa lo w 25 del		42	C	WOSR lo w
	19	L	WOSR+wfa lo w 50		43	G	WOSR+fs cl lo w del
	20	J	WOSR+fs fa lo w del		44	X	wp del
	21	S	WOSR+wp lo w 25		45	R	WOSR+wp lo w 50 del
	22	H	WOSR+fs fa hi w		46	A	WOSR hi n
	23	N	WOSR+wfa lo w 25		47	H	WOSR+fs fa hi w
	24	M	WOSR+wfa lo w 50 del		48	P	WOSR+wp hi w 50

Table 24. Replicated blocks description

	plot	Led	Treatment (led) abbreviation		plot	led	Treatment (led) abbreviation
Block III	49	L	WOSR+wfa lo w 50	Block IV	73	V	wfa del
	50	R	WOSR+wp lo w 50 del		74	P	WOSR+wp hi w 50
	51	Q	WOSR+wp lo w 50		75	U	Wfa
	52	J	WOSR+fs fa lo w del		76	T	WOSR+wp lo w 25 del
	53	H	WOSR+fs fa hi w		77	N	WOSR+wfa lo w 25
	54	I	WOSR+fs fa lo w		78	S	WOSR+wp lo w 25
	55	S	WOSR+wp lo w 25		79	E	WOSR+fs cl lo n
	56	D	WOSR+fs cl hi n		80	X	wp del
	57	N	WOSR+wfa lo w 25		81	I	WOSR+fs fa lo w
	58	X	wp del		82	C	WOSR lo w
	59	E	WOSR+fs cl lo n		83	O	WOSR+wfa lo w 25 del
	60	W	Wp		84	J	WOSR+fs fa lo w del
	61	M	WOSR+wfa lo w 50 del		85	R	WOSR+wp lo w 50 del
	62	K	WOSR+wfa hi w 50		86	M	WOSR+wfa lo w 50 del
	63	T	WOSR+wp lo w 25 del		87	F	WOSR+fs cl lo w
	64	V	wfa del		88	H	WOSR+fs fa hi w
	65	C	WOSR lo w		89	G	WOSR+fs cl lo w del
	66	U	Wfa		90	L	WOSR+wfa lo w 50
	67	B	WOSR hi w		91	K	WOSR+wfa hi w 50
	68	P	WOSR+wp hi w 50		92	D	WOSR+fs cl hi n
	69	O	WOSR+wfa lo w 25 del		93	A	WOSR hi n
	70	G	WOSR+fs cl lo w del		94	B	WOSR hi w
	71	A	WOSR hi n		95	Q	WOSR+wp lo w 50
	72	F	WOSR+fs cl lo w		96	W	Wp

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