

Agroecological practices to enhance resilience of farming systems

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Published in

Frontiers in Agronomy



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ISSN 1664-8714
ISBN 978-2-8325-6581-0
DOI 10.3389/978-2-8325-6581-0

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Agroecological practices to enhance resilience of farming systems

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Citation

Gresta, F., Von Cossel, M., Scordia, D., eds. (2025). *Agroecological practices to enhance resilience of farming systems*. Lausanne: Frontiers Media SA.
doi: 10.3389/978-2-8325-6581-0

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OPEN ACCESS

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RECEIVED 05 June 2025

ACCEPTED 16 June 2025

PUBLISHED 25 June 2025

CITATION

Scordia D, von Cossel M and Gresta F (2025)
Editorial: Agroecological practices to
enhance resilience of farming systems.
Front. Agron. 7:1641522.
doi: 10.3389/fagro.2025.1641522

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Editorial: Agroecological practices to enhance resilience of farming systems

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KEYWORDS

agroecology, resilience, climate change, farming systems, agroecosystem, sustainability

Editorial on the Research Topic

Agroecological practices to enhance resilience of farming systems

1 Introduction

Agroecology traces its origins to the early 20th century, when Basil Bensing coined the terms “agro-ecology” and “agro-ecological research” in 1930 to describe the application of ecological principles to agriculture (Bensing, 1930). Agroecology emerged as a formal discipline through the pioneering work of Tischler in the 1950s–60s, culminating in his seminal book *Agrarökologie* (Tischler, 1965). His research addressed pest management, soil biology, insect biocoenosis, and plant protection, emphasizing ecological processes across both cultivated and non-cultivated landscapes (Wezel et al., 2009). From the 1970s to 1990s, agroecology gained prominence as a response to the environmental and social consequences of the Green Revolution (Gliessman, 2013), with countries in Latin America becoming key hubs for farmer–scientist collaboration on sustainable alternatives (Altieri, 1996). Today, agroecology refers to either a scientific discipline, an agricultural practice, or a political and social movement (Wezel et al., 2009).

Climate change and the overexploitation of natural resources in conventional or industrial agriculture are compromising the sustainability of agroecosystems, undermining future food security, agricultural resilience, and planetary health (van Vuuren et al., 2025). The FAO’s 10 Elements of Agroecology (FAO, 2018) and the HLPE’s 13 Agroecological Principles (HLPE, 2019) are complementary frameworks developed to guide the transformation of food and agricultural systems toward sustainability and resilience, grounded in agroecological approaches. These frameworks translate ecological principles into practical strategies, emphasizing diversity, co-creation, resource efficiency, and equity, enabling farmers to enhance resilience, reduce external input reliance, and support local food systems.

Therefore, in contrast to conventional or industrial agriculture, agroecology offers a holistic framework that integrates ecological, social, and human dimensions across temporal and spatial scales (Wezel et al., 2020). By leveraging synergies among natural processes and stakeholder knowledge, agroecology enhances the adaptive capacity of farming systems and guides transitions toward sustainable and climate-resilient food systems.

This Research Topic addresses these challenges by presenting empirical and conceptual insights demonstrating the effectiveness of agroecological practices in building agroecosystem

resilience and mitigating the impacts of climate change. The selected manuscripts from diverse geographic regions (Figure 1) converge around three major themes: (i) Multicriteria analysis and identification of research gaps to improve the implementation and scaling of agroecology practices; (ii) Crop diversification strategies that contribute to improved productivity, ecosystem services, and climate adaptability; (iii) Soil management and diversification approaches that restore soil health, support carbon storage, and improve nutrient cycling.

Collectively, these contributions underscore the interdisciplinary nature of agroecological research, demonstrating how progress in agroecology depends on the integration of agronomy, ecology, socioeconomics, and participatory governance.

2 Multicriteria analysis of agroecology

Multicriteria analyses and original studies have assessed the current state of agroecology and its potential to enhance system resilience. Altieri et al. highlighted the limits of agroecology adaptation under increasingly severe climate events, noting that smallholder practices like intercropping, agroforestry, mulching, and organic amendments improve drought resilience but may be insufficient under prolonged stress. They emphasized the need for strategies that sustain productivity during extended droughts, alongside tools to assess resilience, while acknowledging the importance of broader interventions such as watershed restoration and policy support.

von Cossel et al. synthesized meta-analyses on agroecology, focusing on crop diversification and soil management. Key practices included agroforestry, cover cropping, intercropping, mixed varieties and use of local varieties, as well as green

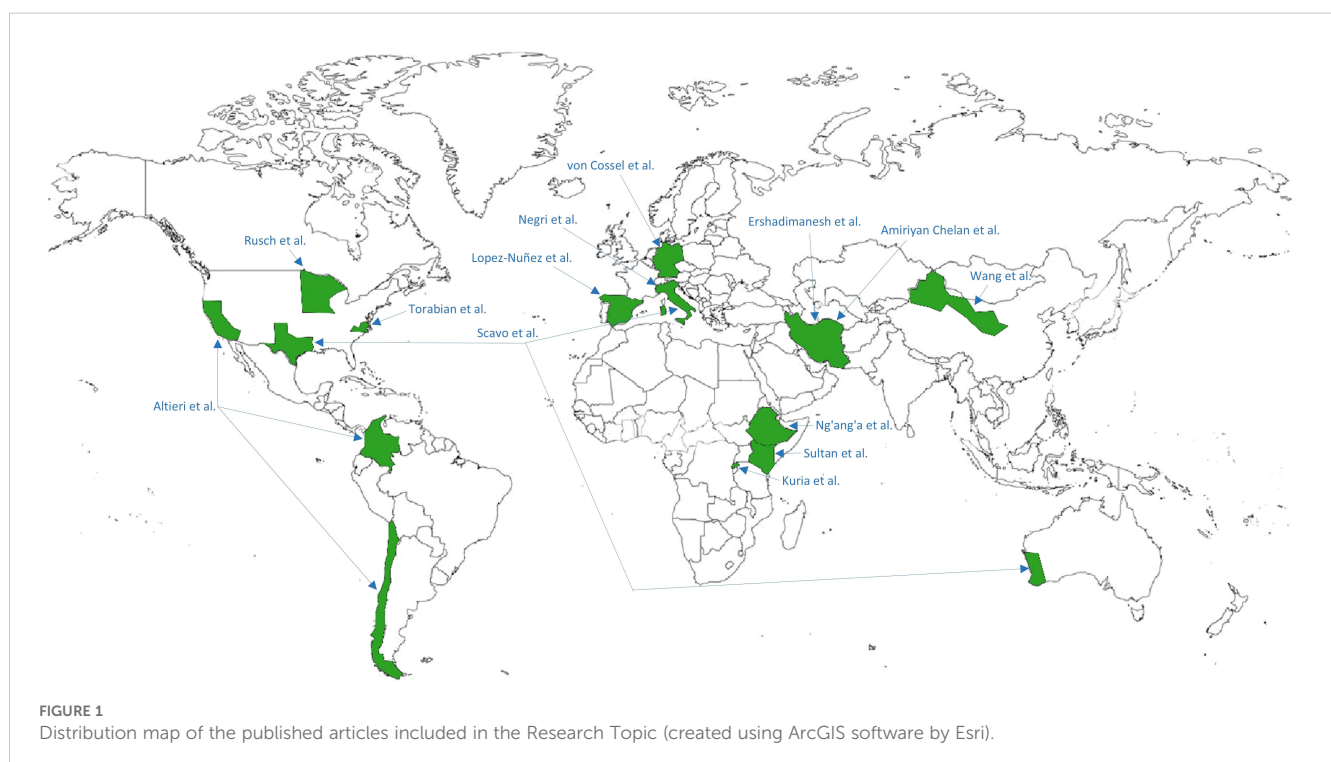
manures, mulching, no-till, and organic inputs. Outcomes varied by site, reflecting complex ecological and socio-economic interactions. The authors proposed a systems-based approach integrating crop-livestock dynamics and circular economy principles. Further research and long-term monitoring should address crop and soil diversification jointly to enhance resilience and support farmer-oriented solutions.

Negri et al. compared agroecology responses in California and Italy, regions facing increased temperatures, erratic rainfall, and declining yields in specialty crops. Practices such as cover cropping, diversification, and precision irrigation can improve soil health and water use, but tailored strategies, policy support, and international cooperation were deemed critical for effective adaptation.

Agroecology transitions in Western Rwanda using longitudinal data from 150 farmers (1995–2015) were examined by Kuria et al. Policy shifts and land scarcity led to the loss of low-value crops, reducing diversity and increasing food insecurity in 83% of households. Though perennial crops buffered seasonal hunger, on-farm food self-sufficiency declined from 10.1 to 6.6 months. The study identified seven agroecology principles as key to resilience, underscoring the need for context-specific, inclusive policies grounded in local knowledge.

3 Crop diversification strategies

Here, annual grain legumes, annual and perennial cereals, and key agroecology practices were studied. In Tanzania, Lelei et al. evaluated integrated soil fertility management in degraded maize systems. Combining lime with mineral fertilizers, i.e., nitrogen (N), phosphorus and potassium, improved yields and soil quality, while



lime with manure proved more cost-effective and sustainable, supporting smallholder livelihoods.

Rusch et al. studied the perennial grass, intermediate wheatgrass (*Thinopyrum intermedium*), in Minnesota over four years. The dual-purpose grain-and-grazing system matched or surpassed the combined yields of grain and straw after year 2 and provided high-quality forage (protein: 140–150 g kg⁻¹). Though initial grain returns were lower, diversified forage income and peak productivity in year 3 suggest that delayed grazing could optimize profitability.

Ng'ang'a et al. assessed the profitability and risk of agroecology practices among wheat farmers in Ethiopia. A cost-benefit analysis showed certified seeds were most profitable, followed by optimized fertilizer use and drainage (net present value: 2531, 2371, 2099 US\$ ha⁻¹, respectively). Despite favorable returns, adoption depends on social and behavioral factors, warranting further research to promote agroecology practices better.

At Virginia State University, varietal performance and planting date effects on faba bean were evaluated for rotation potential. Under current conditions, fall planting with specific varieties produced 58% more branches, double the grain yield, and heavier seeds than spring planting (Torabian et al.). Insight into nutrient components and crop succession is needed to optimize cropping systems, including faba bean.

Ershadimanesh et al. examined source–sink dynamics in bread wheat through defoliation treatments ‘removal of the flag leaf’ (RFL), ‘removal of all leaves’ (RAL), and ‘removal of the upper half of the spikes’ (RHS) under irrigated and rainfed conditions. Drought reduced grain weight per spike (18%) and yield (25%). Defoliation reduced grain weight by 6.7–12.3%, with RFL and RAL enhancing stem and spike remobilization. The RHS treatment showed stronger sinks in vegetative organs than grains but stimulated remobilization. Enhancing both photosynthetic capacity and sink strength is critical to improve yield.

4 Soil management strategies

Rhizobium bacteria, arbuscular mycorrhizal fungi (AMF), growth-promoting bacteria (GPB), mulching, and integrated fertilizers to enhance crop yield and soil health were studied.

In East Azarbaijan, Amiriyan Chelan et al. evaluated the effects of AMF, GPB, and chemical fertilizer on fenugreek intercropped with Moldavian balm. Intercropping (100:50 ratio) with AMF+GPB significantly improved oil yield, fatty acid content, and land equivalent ratio. The treatment also increased anthocyanins, flavonoids, mucilage, and linoleic acid by up to 15.2%, supporting its suitability for sustainable systems.

Scavo et al. assessed biological N fixation in five Mediterranean forage legumes using three rhizobia inoculants, i.e., Australian granular, Australian peat, and American peat, at standard and double doses. Australian granular performed best overall, while American peat was effective only at higher doses. Double-dose inoculation notably enhanced nodulation and N-fixation,

highlighting the need for tailored legume–inoculant combinations to reduce fertilizer dependence.

Lopez-Nuñez et al. tested chitosan for managing soil fungi in persimmon plots under conventional and ecological systems. In pots, chitosan reduced soil pH, conductivity, and cation exchange capacity without affecting soil respiration. In the field, chitosan coacervates boosted the beneficial fungus *Purpureocillium* (50-fold) and suppressed pathogens like *Fusarium* (–50%) and *Alternaria* (–20%). Microbial network analysis showed enhanced roles for nematophagous fungi, affirming chitosan's contribution to soil health.

On the Loess Plateau, Wang et al. conducted a 3-year study on maize systems. High-density planting combined with fertilization and mulching increased yields and water use efficiency by 34–56% over basic farming practices. It furthermore outperformed controls in photosynthetic rate, leaf area index, chlorophyll content, and root growth, underscoring the value of integrated practices in semiarid agriculture.

Author contributions

DS: Conceptualization, Data curation, Project administration, Supervision, Visualization, Writing – original draft, Writing – review & editing. MC: Project administration, Validation, Visualization, Writing – review & editing. FG: Project administration, Supervision, Validation, Writing – review & editing.

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OPEN ACCESS

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RECEIVED 28 February 2024

ACCEPTED 08 May 2024

PUBLISHED 05 June 2024

CITATION

Ershadimanesh K, Siosemardeh A and Hoseeinpanahi F (2024) Evaluation of source–sink manipulation through defoliation treatments in promising bread wheat lines under optimal irrigation and rainfed conditions.

Front. Agron. 6:1393267.

doi: 10.3389/fagro.2024.1393267

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Evaluation of source–sink manipulation through defoliation treatments in promising bread wheat lines under optimal irrigation and rainfed conditions

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The source–sink (S-S) ratio during the grain-filling period is crucial for wheat crop yield. The aim of this study was to investigate the relative sensitivity of grain yield in response to treatments of S-S ratio changes to determine the extent of S-S limitation during grain filling in modern wheat genotypes. The S-S manipulation treatments included four levels: check (CH), removal of flag leaf (RFL), removal of all leaves (RAL), and removal of the upper half of the spikes (RHS). The results showed significant differences between genotypes ($p_b < 0.001\%$) in all traits. Drought stress decreased grain weight per spike (GWS) (g) and grain yield (GY) (kg/ha) by 18% and 25%, respectively. The average reduction in GWS under irrigation and rainfed conditions was 8.25% and 6.71% for RFL and 12.25% and 11.15% for RAL, respectively. By RFL and RAL, increasing the remobilization from the stem and spike straw helped to reduce the effects of source limitation. Also, by RHS, the reduction in photosynthetic materials production in both conditions was only equivalent to 38% and 29% of the expected values, respectively, which shows the presence of strong sinks in vegetative organs (stem and spike) compared to grains. Vegetative organs seem to have a larger sink for the uptake of photosynthetic materials than grains when the source–sink ratio increases. However, high-yield genotypes showed more severe source limitation, while low-yield genotypes showed more relative sink limitation. Overall, to increase the yield potential in high-yielding genotypes, photosynthetic sources and sinks in low-yielding genotypes should be improved.

KEYWORDS

chlorophyll, grain yield, photosynthetic materials, remobilization, source and sink limitation, water soluble carbohydrates

1 Introduction

Bread wheat (*Triticum aestivum* L.) is one of the most important crops in the world, and its productivity has to be increased significantly to feed the growing world population, which is expected to reach over 9 billion by 2050 (CIMMYT, 2017; Alonso et al., 2018). The cultivated area of this plant in the world is 221 million hectares, and its production amount is 771 million tons (FAO, 2021). In major wheat-growing areas of the world, its productivity is adversely affected by various abiotic stresses, and among them, drought is the major abiotic stress causing serious damage (Saradadevi et al., 2017). In particular, terminal drought refers to the drought after anthesis, and it usually causes grain weight reduction and yield loss (Reynolds et al., 2005). The scenarios of terminal drought also alter the balance between sources and sinks of assimilation and consequently depress the rate and duration of grain filling and sink capacity (Ovenden et al., 2017). In this regard, physiological traits and processes related to drought resistance, including the source–sink (S–S) photosynthetic capacity, should be more accurately evaluated to be used in the breeding process of drought-tolerant cultivars. The growth and grain filling in wheat are controlled by the relationships between source strength and sink capacity (Foulkes et al., 2011). Knowing the physiological relationships between S–S can help to select and improve wheat grain yield (Maydup et al., 2013).

Source tissues are generally responsible for acquiring resources from the external environment, although the remobilization of stored resources may also turn a sink into an internal source. The term source strength refers to the net rate of uptake (mol/s) of a particular resource from the external environment, as seen in Equation 1:

$$\text{Source strength} = \text{source size} \times \text{source activity} \quad (1)$$

where source size refers to the total biomass of source tissue (g), and source activity is the specific uptake rate of the resource ($\text{mol g}^{-1} \text{s}^{-1}$). Also, sink tissues are net receivers of resources from source tissues. The term sink strength refers to the net rate of uptake (mol/s) of a particular resource by a defined tissue within the plant, as seen in Equation 2:

$$\text{Sink strength} = \text{sink size} \times \text{sink activity} \quad (2)$$

where sink size is the total biomass of sink tissue (g), and sink activity refers to the specific uptake rate of the resource ($\text{mol g}^{-1} \text{s}^{-1}$). Source tissues thus take up environmental resources and export them to sinks (White et al., 2016). The leaf is the major organ involved in light perception and the conversion of solar energy into organic carbon (Du et al., 2019). The flag leaf is the main component of the canopy in the middle and late growth stages of winter wheat (Liu et al., 2021) and is an important organ that determines the grain-filling rate and the final yield (Vicente et al., 2018). The contribution rate of flag leaves to daily photosynthetic products varies from 50% to 60% (Towfiq et al., 2015), while its defoliation generated grain yield losses of 18% to 30% (Ma et al., 2021). In wheat, the defoliation of the flag leaf blade increased the contribution of assimilates to the grain from the stem and the chaff under normal conditions (Alvaro et al., 2008), and the removal of these affected the grain yields under normal or water-limiting conditions (Cruz-Aguado et al., 1999). Chlorophylls and carotenoids are photosynthetic pigments capable of absorbing light,

transmitting energy to the photochemical and biochemical phases of photosynthesis, and accumulating chemical energy that is stored as sugar (Bojović and Stojanović, 2005). Determination of chlorophyll content as an indirect method of estimating the productivity of vegetation represents a good way to gain an understanding of the photosynthetic regime of plants (Niroula et al., 2019).

The sink size of developing yield organs is determined by the number of spikes per unit area, grain number per spike (GNS), and the specific sink size per grain. Source size is related to the production of photo-assimilates, namely, the size, photosynthetic capacity, and duration of leaf area, which drives spike development and grain filling (Jagadish et al., 2015). Grain yield is often limited by sink capacity or lack of photo-assimilates (Maydup et al., 2013). One of the ways to achieve high yield in wheat genotypes is to allocate more photo-assimilates to economic sinks (grains) (Felekori et al., 2014). Manipulation of source strength and sink capacity has been investigated in several studies to determine the mechanisms controlling grain yield. In a balanced situation between S–S, the highest grain yield is produced (Borras and Salfer, 2004). In this regard, various treatments such as removal of leaves and shading indifferent light intensities and for different periods (Wang et al., 2003), increasing the concentration of carbon dioxide (Manderscheid et al., 2003), and removal of spikelets and grains in different parts of the spike (Cruz-Aguado et al., 2000) have been used to investigate S–S relationships in wheat, leading to different results by researchers. The researchers studied the effects of removing the flag leaf and removing the upper half of the spike in 24 durum wheat varieties (both modern and old cultivars). They observed that the treatment of removing half of the spike increased the weight of the remaining grains, indicating a limitation in the supply of photo-assimilates during grain filling under normal conditions (Alvaro et al., 2007). The increase in grain weight in response to the decrease in sink ratio indicates that grains have not reached their maximum growth under normal conditions due to insufficient photosynthesis (Saeidi et al., 2011). In addition, other experiments also show that most improved wheat genotypes have resource limitations.

Depending on the environmental conditions, genotypes have different resource limitations, and it seems that examining the degree of limitation in wheat genotypes in a region shows the degree of compatibility of each genotype with that environment (Ahmadamini et al., 2011). Also, some researchers indicate both S–S limitations in wheat (Abdoli et al., 2013). When the S–S ratio decreases, sink-limited cultivars should be less affected than source-limited cultivars. In other words, defoliation reduces both traits of grain growth rate and grain weight of cultivars, but the relative reduction will be greater for cultivars with limited resources (Abdoli and Saeidi, 2013). Artificial defoliation in wheat may change the photosynthetic characteristics of the remaining tissues (Zhenlin et al., 1998). Researchers reported that after anthesis, source limitation by defoliation of winter wheat increased the net photosynthesis rate and chlorophyll content of wheat leaves (Zhu et al., 2004; Joudi et al., 2006).

The accumulation potential of storage materials in the stems and the rate of remobilization of these materials from the stem to the growing grains are two crucial characteristics in wheat grain yield and related to S–S relationships, which determine the final

grain yield under environmental stress (Najafian and Shabani, 2010). In resistant cultivars, remobilization from stem nodes is more significant, especially under drought stress conditions (Saeidi et al., 2012). Under terminal drought stress, stem carbohydrate reserves become the major source of grain filling as leaf photosynthesis ceases (Zhang et al., 2015). These reserves are water-soluble carbohydrates (WSCs), mainly consisting of fructan and glucose, fructose, and sucrose as well as various oligosaccharides (Joudi et al., 2012). The amount of accumulation and remobilization of carbohydrates in the wheat stem can be estimated either by monitoring the changes in stem dry weight (Ma et al., 2014; Thapa et al., 2022) or by measuring the stem WSC content (Liu et al., 2020). Drought stress significantly accelerates the remobilization of pre-anthesis stem water-soluble carbohydrate reserves during the period of grain filling (Liu et al., 2020). The lower grain weight reduction per spike in some genotypes in response to source reduction could be stimulation and remobilization of more storage materials from stem to grains, which partially compensates for yield reduction (Khan et al., 2002). The researchers reported that genotypes with a higher remobilization rate were less affected by drought stress during the final growing season (Yang and Zhang, 2006), and severe drought stress increased the remobilization rate to grow grains due to early maturity and dropping of lower leaves (Bagherikia et al., 2017). In this regard, the researchers obtained a positive and significant correlation between the amount of remobilization and grain weight under stress conditions (Papakosta and Gayanas, 1991).

Most studies to investigate the relationship between S-S in wheat have been conducted on a limited number of cultivars and still need a preliminary evaluation of the relative limitations of S-S in the investigated genotypes. In continuation of the previous research, it is necessary to first identify the relative degree of S-S limitation based on morpho-physiological traits in a broader range of genotypes and then investigate more precise compensatory mechanisms in a smaller range of genotypes. The present study was conducted in order to investigate the S-S relationship and to evaluate the relative sensitivity of grain yield, in response to treatments of reduction S-S ratio, in two conditions of optimal irrigation and rainfall. Also, in order to more closely investigate the S-S relationship, the remobilization values of stem and spike straw affected by the S-S limitation treatments were investigated.

2 Materials and methods

2.1 Plant materials

In the first year, 50 advanced bread wheat lines, the result of Icarda and Simit breeding programs, and six conventional bread wheat cultivars (Baharan, Pishgam, Pishtaz, Sirvan, Heydari, and Miham) as check were grown under irrigation conditions (Table 1). In the second year, based on the experimental objectives and available diversity, 11 genotypes were selected including nine advanced lines (including three lines with source limitation, three lines with sink limitation, and three intermediate lines with both S-S

limitations) to create genetic diversity and two conventional cultivars (Pishgam and Baharan) as check (Table 2). Lines with source limitation were selected based on the flag leaf area, soil plant analysis development (SPAD) values, and date to anthesis traits; lines with sink limitation were selected based on the GNS, grain weight, grain-filling duration, and grain-filling rate traits; lines with both S-S limitations were selected with intermediate traits (Table 1). These lines and cultivars were chosen because they have higher yields than other cultivars of different plant types (Table 2).

2.2 Experimental design and field management

This experiment was conducted during the 2017–018 and 2018–2019 cropping years at the Islamabad-e-Gharb Agricultural Research Station (latitude 34°8' North, longitude 47°26' East, altitude 1,346 m above sea level). The average annual rainfall was 468 mm, and the average annual temperature was +13°C. The climatic characteristics of the experiment sites are listed in Figures 1A, B. In the first year of the experiment, the desired genotypes were investigated in the format alpha-lattice design in two replicates under irrigation conditions to select suitable genotypes. The genotypes were cultivated on November 5, 2017, in an experimental planter, and the seed rate was 400 seeds/m². Plots were 3 m long and 1.2 m wide (3.6 m² total plot area) with six rows in each spaced 0.2 m apart. In the second year, 11 selected genotypes including lines 12, 15, 24, 26, 27, 28, 33, 44, and 48, and two cultivars (Pishgam and Baharan) as check were planted (Table 2). The experimental design was split-plot in a randomized complete block design (RCBD) with three replicates in two separate sites, including i) irrigation and ii) rainfed conditions, using macro plots of 10 m long and 1.2 m wide (12 m² total plot area) with six rows in each spaced 0.2 m apart, assigning the 11 genotypes to the main plots and the four S-S treatments (CH, RFL, RAL, and RHS) to the subplots. The genotypes were planted in an experimental planter on November 11, 2018, and in both conditions, the seed rate was 400 seeds/m². Irrigation was done with a fixed classical system, and the irrigation cycle was considered once every 6–8 days based on the conventional agriculture of the region. In rainfed conditions, no irrigation was done during the cropping season, and the amount of rainfall is shown in Figure 1B. The experimental field in the previous crop year was fallow, and the soil type was clay-loam. The physical and chemical characteristics of the experiment site are shown in Table 3. The amount of chemical fertilizers was determined and applied based on the soil test (Table 3), including 200 kg N/ha using urea (46% N) with 50 kg N/ha in sowing, and an additional 150 kg N/ha was applied at the jointing stage. P and K were applied as basal fertilizers with 100 kg P/ha as triple superphosphate (46% P₂O₅ and 15% Ca) and 50 kg K/ha as potassium sulfate (K₂O51% and S 17%). Common herbicides applied to weed control include 2,4-D herbicide for eliminating broad-leaf weeds and clodinafop-propargyl for eliminating narrow-leaf weeds, and for pest control, chlorpyrifos-ethyl insecticide (1,500 mL/ha) was used twice during the crop cycle.

TABLE 1 The mean comparisons of grain yield (GY), grain number per m² (GN), grain number per spike (GNS), grain weight per spike (GWS), thousand-grain weight (TGW), spike length (SL), flag leaf area (FLA), flag leaf area per each spike grain (FLAS), date to anthesis (DA), date to maturity (DM), grain-filling duration (GFD), grain-filling rate (GFR), and soil plant analysis development (SPAD) value (SP) in the first year under optimal irrigation conditions.

Gen no.	GY (t/ha)	GN (n)	GNS (n)	GWS (g/spike)	TGW (g)	SL (cm)	FLA (cm ²)	FLAS (cm ²)	DA (day)	DM (day)	GFD (day)	GFR (mg/day)	SP (%)
1	7.57	14,466	41.5	2.14	52.33	7.86	12.08	0.29	172.5	208	35.5	0.0015	42.4
2	6.1	13,917	37.5	1.64	43.83	8.76	10.65	0.28	171	208.5	37.5	0.0012	42.5
3	7.7	13,363	40.1	2.29	57.62	10.56	12.57	0.31	172.5	209	36.5	0.0016	42.55
4	7.6	15,033	47.2	2.39	50.49	8.86	12.57	0.27	172.5	210	38	0.0013	40.9
5	6.56	14,738	34.9	1.6	44.51	8.16	10.07	0.29	172.5	206	33.5	0.0013	42.15
6	8.52	14,153	33.7	2.03	58.2	8.15	8.66	0.23	168.5	208.5	40	0.0015	41.35
7	7.87	16,097	39.7	1.95	48.89	8.31	11.56	0.29	172	207	35	0.0014	42.35
8	8	13,894	36.1	2.08	57.58	9.96	10.43	0.29	170	207.5	37.5	0.0015	43.25
9	7.49	14,944	35.3	1.75	50.12	8.71	11.08	0.31	165.5	206	40.5	0.0012	37.95
10	8.18	15,324	39.2	2.09	53.38	8.76	9.43	0.24	171	208	37	0.0014	44
11	7.5	13,871	46.8	2.53	54.07	7.91	12.86	0.27	171.5	207.5	36	0.0015	46.35
12	9.32	19,742	39.4	1.86	47.21	8.06	9.64	0.24	173.5	210	36.5	0.0013	40.95
13	8.9	15,152	40.8	2.38	58.74	8.41	10.87	0.27	170.5	208.5	38	0.0015	40.8
14	8.28	18,359	44.2	1.99	45.1	8.96	14.48	0.33	172.5	210.5	38	0.0012	47.85
15	8.35	17,965	43.4	1.68	46.48	8.16	9.44	0.22	167	205	38	0.0012	42.85
16	9.18	22,174	38.5	1.99	41.4	7.66	8.18	0.21	170.5	208	38.5	0.0011	46.1
17	7.65	15,731	49.2	1.41	48.63	7.76	11.73	0.24	174.5	212	37.5	0.0013	44.4
18	6.55	17,048	45.1	1.73	38.42	8.01	11.39	0.25	173	210	36.5	0.001	43.4
19	8.73	18,363	37.1	1.76	47.54	8.86	10.18	0.27	170.5	208	34.5	0.0013	45.15
20	8.14	18,795	41.4	1.79	43.31	9.21	12.56	0.3	172	208.5	35.5	0.0012	41.3
21	8.57	20,066	48	2.05	42.71	8.86	10.07	0.21	173	209.5	35	0.0012	38.6
22	7.73	13,609	32.2	1.83	56.8	8.51	9.34	0.29	167.5	207	38	0.0014	41.6
23	7.12	12,439	39.1	2.25	57.24	8.11	15.28	0.39	170.5	208.5	37.5	0.0015	44.2
24	8.49	16,218	32.5	1.71	52.35	9.46	13.35	0.41	168.5	207	34.5	0.0012	46.6
25	8.88	19,035	41.3	1.91	46.65	9.31	10.9	0.26	168	207.5	36.5	0.0012	41.9
26	8.05	16,415	31.4	1.74	49.04	8.06	7.82	0.25	162.5	206	41	0.0011	37.15

(Continued)

TABLE 1 Continued

Gen no.	GY (t/ha)	GN (n)	GNS (n)	GWS (g/spike)	TGW (g)	SL (cm)	FLA (cm ²)	FLAS (cm ²)	DA (day)	DM (day)	GFD (day)	GFR (mg/day)	SP (%)
27	7.29	11,707	36.3	2.28	62.27	8.61	8.15	0.22	163.5	206.5	37.5	0.0017	38.7
28	7.49	13,789	41	2.23	54.39	7.56	13.12	0.32	167.5	207	39.5	0.0013	45.5
29	8.24	13,348	32.2	1.97	61.73	9.01	10.51	0.33	171	208.5	37.5	0.0016	44.05
30	7.82	16,603	41.3	1.95	47.1	8.66	13.36	0.32	172.5	211.5	39	0.0012	44.35
31	8.49	16,246	43.4	2.25	52.26	9.01	12.36	0.28	176	212.5	36.5	0.0014	42.15
32	7.96	16,453	38.1	1.84	48.38	8.46	9.69	0.23	167	206.5	32.5	0.0012	43.8
33	7.76	15,702	41.9	2.07	49.42	8.01	8.55	0.28	165.5	210	36.5	0.0014	38.25
34	8.11	16,585	34.8	1.7	48.9	8.66	9.07	0.26	172	206.5	34.5	0.0014	39.45
35	8.07	18,213	43.7	1.94	44.31	8.26	11.25	0.26	173	207.5	34.5	0.0013	41.65
36	8.89	14,794	36	2.17	60.16	8.76	11.84	0.33	171.5	212	40.5	0.0015	42.7
37	5.93	11,153	35	1.86	53.17	8.41	9.58	0.27	170	208	38	0.0014	42.75
38	8.12	18,260	36	1.6	44.47	9.36	13.4	0.37	176.5	212	35.5	0.0012	43.4
39	7.98	17,243	35.3	1.63	46.28	8.46	11.18	0.32	171	208	37	0.0012	42.45
40	8.7	15,204	35.6	2.03	57.22	8.46	9.61	0.27	171	207.5	36.5	0.0016	42.6
41	6.32	12,874	36	1.76	49.09	9.16	10	0.28	171	207.5	36.5	0.0013	41.45
42	7.45	17,210	38.8	1.68	43.29	9.41	11.56	0.3	167.5	207	39.5	0.0011	43.15
43	7.69	17,158	35.8	1.61	44.82	8.56	11.35	0.32	170	206	36	0.0012	42.15
44	9.1	23,680	48.4	2.04	42.23	8.71	12.72	0.26	169	209	40	0.0011	44.75
45	7.98	16,082	39.5	1.95	49.62	7.66	11.19	0.28	167.5	207	39.5	0.0013	44.1
46	8.13	18,003	37.6	1.7	45.16	8.31	11.16	0.3	173.5	208	34.5	0.0013	43.7
47	7.8	16,574	37	1.74	47	7.91	9.64	0.26	171.5	208.5	37	0.0013	45.1
48	8.68	16,809	49.3	2.52	51.64	8.21	12.41	0.25	177	212	35	0.0015	43.1
49	8.83	17,781	39.3	1.95	49.66	8.76	14.66	0.37	170.5	207.5	37	0.0013	45.6
50	9.6	17,195	38.2	2.14	55.83	10.26	10.45	0.27	175	210.5	35.5	0.0016	42.25
Baharan	9.21	19,037	37.3	1.8	48.38	7.66	8.64	0.23	172.5	207.5	35	0.0014	42.95
Sirvan	9.53	18,222	38.8	2.03	52.3	8.01	10.75	0.28	170.5	210.5	40	0.0013	44.45
Haidari	9.86	20,877	43	2.05	47.23	8.71	11.1	0.26	175	213	38	0.0012	42.95

(Continued)

TABLE 1 Continued

Gen no.	GY (t/ha)	GN (n)	GNS (n)	GWS (g/spike)	TGW (g)	SL (cm)	FLA (cm ²)	FLAS (cm ²)	DA (day)	DM (day)	GFD (day)	GFR (mg/day)	SP (%)
Pishgam	10.36	23,370	56.2	2.49	44.33	8.11	15.52	0.28	176	213.5	37.5	0.0012	41.3
Pishtaz	10.21	20,956	34.9	1.7	48.72	10.31	10.55	0.3	175	212.5	37.5	0.0013	43.3
Mihan	9.26	20,351	52.7	2.37	45.01	9.21	15.25	0.29	178	213.5	35.5	0.0013	42.15
Average	8.17	16,650	39.8	1.95	49.8	8.61	11.26	0.28	171.27	208.82	37.11	0.0013	43.11
LSD 5%	0.84	1,937	5.23	0.408	9.39	1.29	2.08	0.023	1.87	1.97	3.88	0.0003	3.44
Average SE of mean	± 0.296	± 94.68	± 1.84	± 0.14	± 3.31	± 0.45	± 0.73	± 0.018	± 1.19	± 0.69	± 1.36	± 0.0001	± 1.21

LSD, least significant difference.

2.3 Trait measurements and growth analysis

In the first year, the grain yield and its components were recorded at maturity. All plants in each plot (3.6 m²) were harvested to determine grain yield (t/ha). Grain number per spike and grain weight per spike were determined by randomly sampling 10 spikes from each plot. Thousand-grain weight was determined from three subsamples of random 100 grains, and grain number was calculated as the ratio between grain yield and thousand-grain weight. Sink capacity (SICA) was calculated as the product of grain number and potential grain weight (Alonso et al., 2018). The date to anthesis and date to maturity were calculated based on the number of days from planting to anthesis (DC65) and planting to maturity stages (DC95; Zadoks et al., 1974). The grain-filling duration was calculated based on the days between anthesis and maturity. The grain-filling rate was calculated based on the weight of a single grain divided by the grain-filling duration (Wych et al., 1982). Spike length was measured based on the average of five spikes using a ruler. The area of the flag leaf was measured using a scanner as well as ImageJ and Photoshop software. All SPAD measurements were taken using a SPAD-502 PLUS chlorophyll meter (Konica Minolta Sensor, Osaka, Japan). SPAD values of the flag leaf in 50 lines and six cultivars were measured 15 days post-anthesis. The SPAD readings were obtained at the upper, middle, and lower positions of each lamina. Five laminae were measured in each plot, and these values were averaged. According to the above traits and to achieve enough diversity between the genotypes, nine lines and two cultivars (checks) were selected for cultivation in the next year (Table 2). In the second year, to measure the grain yield (GY) (kg/ha), the whole plot was harvested. Also, the desired traits include i) grain weight per spike (GWS), ii) GNS, iii) stem weight (SW), and iv) spike straw weight (SSW). Four S-S treatments were applied to the main shoot, i.e., i) check (CH), ii) removal of flag leaf (RFL), iii) removal of all leaves (RAL), and iv) removal of the upper half of the spikes (RHS); 50% of upper spikelets of the spike were removed by cutting with scissors (Serrago et al., 2013). These treatments were performed 15 days after anthesis (DC75; Zadoks et al., 1974) when the grain number (Abbate et al., 1997) and potential grain weight were mainly defined (i.e., the sink).

In this regard, 10 shoots (main stem) were randomly selected, and the above treatments were applied 15 days after anthesis simultaneously for maximum accumulation of storage materials in the stems and spikes. The samples were partitioned into different organs, including stems and spikes (straw and grains), and oven-dried at 75°C for 48 hours until a constant weight was attained. In this regard, to make the same observations in the data and figures, GWS, GNS, and SSW trait values were doubled in the RHS treatment. Also, in maturity, changes in the SW and SSW were measured, and based on this, remobilization values affected by S-S manipulation treatments were calculated. In this regard, remobilization values were measured according to the reduction in stem and spike straw dry weight from 15 days after anthesis to maturity compared to the check. The decrease in the stems or spikes straw dry weight in the source manipulation treatments (RFL and RAL) was considered the increase in remobilization value affected

TABLE 2 Pedigree, selection history, and grain yield (t/ha) of evaluated lines in the first and second years under irrigation conditions.

Gen. no in the first year	Amount of yield in the first year (t/ha)	Amount of yield in the second year (t/ha)	Gen. no. in the second year	Pedigree and selection history
Pishgam	10.4	8.1	1	Bkt/90-Zhong87
Baharan	9.2	7.6	2	KAUZ/PASTOR//PBW343 CMSS00M02401S-030M-030WGY- 030M-18M-0Y
33	7.8	6.7	3	TRAP#1/BOW//PFAU/3/MILAN/4/ ETBW 4922/5/PFAU/MILAN ICW08-50397-6AP-0AP -040SD- 4SD -0SD
26	8.1	6.0	4	ZARAF-5/FLAG-6//MILAN/ PASTOR ICW08-50324-1AP-0AP -040SD- 6SD -0SD
27	7.3	7.0	5	WBL1*2/BRAMBLING/3/OPATA/ RAYON//KAUZ ICW08-00280-8AP-0AP -040SD- 3SD -0SD
15	8.4	7.4	6	P1.861/RDWG/4/SERI.1B//KAUZ/ HEVO/3/AMAD AISBW05-0182-5AP-0AP-0AP-1AP- 1AP-0AP-0TR
24	8.5	7.1	7	MEX94.27.1.20/3/SOKOLL//ATTILA/ 3*BCN/4/ZAFIR-3 ICW08-00220-4AP-0AP -040SD- 7SD -0SD
28	7.5	6.5	8	CHAMRAN/4/OPATA/BOW//BAU/ 3/OPATA/BOW/5/SAMIRA-9 ICW08-50008-21AP-0AP -040SD- 2SD -0SD
48	8.7	7.6	9	TUJAR ICW06-50207-11AP-0AP-0AP -03 SD
44	9.1	7.4	10	PFAU/MILAN//FUNG MAI 24/3/ ACHTAR/INRA 1764 ICW08-00196-11AP-0AP -040SD- 1SD -0SD
12	9.3	8.1	11	VEE/PJN//2*KAUZ/3/SHUHA-4/ FOW-2 ICW06-00836-11AP-0AP-0AP-7AP- 0AP-0TR

by source size reduction. Also, the increase in SW or SSW in the sink manipulation (RHS) treatment was considered a reduction in remobilization values affected by sink size reduction. The amount of accumulation and remobilization of carbohydrates in wheat stem was measured by measuring the WSC content (Liu et al., 2020). Accordingly and based on the literature (Ma et al., 2014), the amount of remobilized WSCs was calculated as follows: WSC remobilization of the stem = the maximum WSCs of the stem at 10 days after anthesis minus the WSCs of the stem at maturity. Also, stem remobilization efficiency was estimated using the proportion (%) of the mobilized WSCs relative to the maximum weight of that segment. The following formula was used in order to calculate the amount of source limitation in RHS treatment (Modhej, 2001).

$$SL = \left[\frac{(a)}{(b)} - 1 \right] \times 100 \quad (3)$$

In this formula, SL (%), a, and b are the source limitation percentage, the average weight of the spike in the halved spikes, and the average weight of the spike in the check, respectively. Also, the following relationship was used to calculate the role and influence of the flag leaf and the whole leaves in filling the grains.

$$EL = \left[\frac{(c-d)}{(c)} \right] \times 100 \quad (4)$$

In this formula, EL (%), c, and d are the role of leaves (flag leaf or total leaves) in grain-filling percentage, the average spike weight

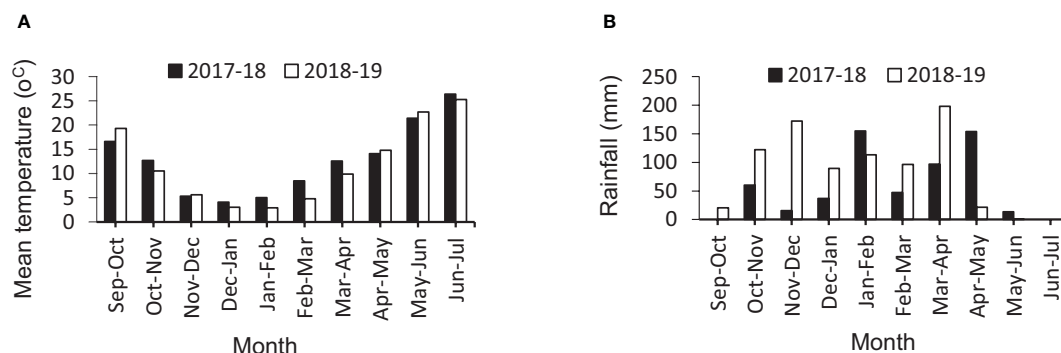


FIGURE 1

Climate conditions. (A) Changes of temperature in two cropping years, 2017–2018 and 2018–2019. (B) Changes of rainfall in two cropping years, 2017–2018 and 2018–2019.

in check, and the average spike weight in defoliated plants (RFL and RAL treatments), respectively. Also, chlorophyll contents, including chlorophyll *a*, chlorophyll *b*, total, and carotenoids, were measured 15 days after anthesis on flag leaf samples based on the method (Arnon, 1967).

2.4 Statistical analysis

In the first year, the studied genotypes were investigated in the format alpha-lattice design and with ALPHA software in two replications under irrigation conditions. In the second year, Statistical Analysis System (SAS ver 9.1) software was used to perform analysis of variance (ANOVA), significance analysis, and Pearson's correlation analysis. The statistical comparisons are indicated by asterisks in the results as significant at the 0.05 (*) and 0.01 (**) probability levels. Mean comparisons among cultivars and S-S manipulations were performed using least significant differences (LSDs) and calculated at the 5% probability level. Finally, the graphs were drawn using EXCEL software.

3 Results

3.1 Selection of genotypes according to yield and morphophysiological traits in the first year

In the first year of the experiment, the effect of cultivar on yield and morphophysiological traits was significant (data not shown). According to the observed diversity of genotypes regarding the above traits, nine out of 50 lines and two out of six cultivars were selected to evaluate the relationship between S-S in the second year (Tables 1, 2). Finally, according to the grain yield and morphophysiological traits, three lines with source limitation (lines 33, 26, and 27), three lines with sink limitation (lines 15, 24, and 28), and three intermediate lines with both relative S-S

limitations (lines 48, 44, and 12) were selected. Also, among the conventional cultivars, the two cultivars as check with the highest yield (Pishgam) and the lowest yield (Baharan) and the diversity of morphophysiological traits were chosen for evaluation along with nine selected lines. The general status of the assessed traits of 11 selected genotypes compared to all evaluated genotypes is shown in Tables 1, 2.

3.2 Grain yield and grain number per spike

The data variance analysis showed that the effects of genotype and S-S manipulation on all evaluated traits were significant under irrigation and rainfed conditions (data not shown). Also, drought stress (rainfed conditions) reduced GY (25%), GNS (12%), GWS (18%), SSW (9%), and SW (18%) compared to irrigation conditions (Table 4). Researchers reported that drought stress in the pre-reproductive stage mainly inhibits the formation of wheat grain number per spike but has little impact on spike number and thousand-grain weight (Zhang et al., 2020; Gao et al., 2023). The GY varied in irrigation conditions at 5,949 to 8,133 and rainfed conditions at 4,550 to 6,587 kg/ha, and the results showed that genotypes with high yield in irrigation conditions had a higher yield in rainfed conditions (Figures 2A, B). The range of variation in the GNS was 28 to 65 and 25 to 47 in irrigation and rainfed conditions, respectively, depending on cultivar, treatment, and environment (Figures 3B, 4B). Genotypes 1, 9, 3, 10, and 11 had the highest GNS, and genotypes 4, 5, 2, and 7 had the lowest GNS in both irrigation and rainfed conditions (Table 4). Also, range of variation in GWS was 1.38 to 2.04 (g spike⁻¹) and the most reduction in GNS was related to the RAL treatment in rainfed conditions (Figures 4A, 5B). A significant positive relationship ($r = 0.637^*$) was observed between grain yield and GNS in rainfed conditions (Table 5). In the RFL treatment, the average decrease in GNS was 8% and 3% in irrigation and rainfed conditions, respectively, and in the RAL treatment, it was 17% and 9%, respectively (Table 6). Furthermore, in most investigated lines, RHS treatment did not affect GNS.

TABLE 3 Soil information (0–20-cm depth) of Islamabad-e-Gharb Research Station (2017–2018 and 2018–2019 cropping years).

Year	Organic carbon (%)	Silt (%)	Sand (%)	Clay (%)	Texture type	pH	N (%)	P (mg/kg)	K (mg/kg)	Cu (mg/kg)	Mn (mg/kg)	Fe (mg/kg)	Zn (mg/kg)
2017–2018	1.29%	57	3.6	39.4	Si-C-L	8.1	0.13	10.35	432	1.66	7.2	6.89	0.78
2018–2019	1.12%	59.7	3.1	37.2	Si-C-L	7.9	0.11	9.85	485	1.84	6.9	6.31	0.74

3.3 Source and sink S-S limitation

Some levels of source limitation were observed from 8.18% to 10.25% on average in all genotypes. Genotypes showed different reactions to defoliation levels (reduction of source strength) in GWS in both experiments. Defoliation treatments caused a significant decrease in GWS, and GWS decreased by 18% under drought stress in rainfed conditions (Table 6; Figure 5A). In the RFL treatment, the reduction of GWS was 8% and 7% in irrigation and rainfed conditions, respectively. Also, genotypes showed different reactions to the RFL, so genotypes 1 and 10 in irrigation conditions and 4 and 11 in rainfed conditions showed the highest reaction (Table 7). Genotypes 1, 5, and 6 in both irrigation and rainfed experiments showed the highest reaction to the RHS treatment, which indicates the amount of source limitation (SL) and the insufficiency of photosynthetic materials in grain-filling duration. High-yielding genotype 1, with 24% SL in irrigation conditions, showed the highest SL among the genotypes (Table 7). The same situation was observed under drought stress; genotypes 1 and 10 had the highest SL and grain yield in rainfed conditions at 31% and 14%, respectively (Table 7). Also, lines 3 and 7 showed the least response to sink reduction in both irrigation and rainfed conditions, which indicates the relative limitation of the sink in them (Table 7). In this research, a significant positive relationship was observed between SL and GY (kg/ha) in irrigation ($r = 0.647^*$) and rainfed ($r = 0.702^*$) conditions and also with GNS in rainfed conditions ($r = 0.692^*$). Furthermore, the positive relationship between SL and GWS is significantly known in irrigation ($r = 0.658^*$) and rainfed ($r = 0.632^*$) conditions (Table 5).

3.4 Stem weight

In the present work, the effects of genotype and defoliation intensities on SW were significant under irrigation and rainfed conditions (data not shown). The SW decreased in RFL and RAL treatments (reduction of source strength), while it increased in RHS treatment (reduction of sink strength) under irrigation and rainfed conditions (Figures 6A, B, 7A, B). Also, the increase in stem remobilization affected by source reduction was calculated by measuring the stem dry weight at maturity in defoliation treatments compared to the check (Table 8). In this regard, the remobilization values from stem to grain in the check (without S-S manipulation) were 0.63 and 0.81 g/stem in irrigation and rainfed conditions, respectively, which were equivalent to 32% and 51%, respectively, of grain weight (Table 4). The increase in stem remobilization (with S-S manipulation) compared to that in the check was 8% (RFL) and 21% (RAL) in irrigation conditions, which was equivalent to 3% and 7% of grain weight, while in rainfed conditions, the increase was 9% (RFL) and 16% (RAL), which was equivalent to 4% and 7% of grain weight, respectively (Table 6).

3.5 Spike straw weight

In this study, the effects of genotype and defoliation intensities on SSW were significant under irrigation and rainfed conditions

TABLE 4A The mean comparisons of grain weight per spike (GWS), grain number per spike (GNS), and spike straw weight (SSW) traits in various genotypes (Factor A) in irrigation (I) and rainfed (R) conditions in second year of experiment.

Genotype	GWS (g)			GNS (g)			SSW (g)		
	(I)	(R)	Change %	(I)	(R)	Change %	(I)	(R)	Change %
Pishgam	2.57 ± 0.039	2.04 ± 0.109	−21	56 ± 1.08	52 ± 1.83	−7	0.74 ± 0.004	0.71 ± 0.020	−4
Baharan	1.80 ± 0.116	1.55 ± 0.067	−14	38 ± 2.33	35 ± 0.33	−8	0.76 ± 0.021	0.71 ± 0.047	−7
3	1.91 ± 0.055	1.74 ± 0.061	−9	52 ± 1.86	44 ± 1.91	−15	0.99 ± 0.012	0.98 ± 0.017	−1
4	1.81 ± 0.081	1.41 ± 0.048	−22	36 ± 0.87	33 ± 1.23	−8	0.70 ± 0.011	0.55 ± 0.017	−21
5	1.87 ± 0.038	1.48 ± 0.041	−21	42 ± 1.16	34 ± 1.13	−19	0.73 ± 0.016	0.70 ± 0.013	−4
6	1.85 ± 0.049	1.44 ± 0.025	−22	44 ± 0.73	40 ± 1.18	−9	0.66 ± 0.016	0.54 ± 0.014	−18
7	1.91 ± 0.038	1.38 ± 0.031	−28	36 ± 0.91	29 ± 0.71	−19	1.09 ± 0.037	0.76 ± 0.017	−30
8	1.74 ± 0.062	1.44 ± 0.057	−17	44 ± 1.13	35 ± 1.46	−20	0.72 ± 0.009	0.71 ± 0.030	−1
9	2.16 ± 0.013	1.92 ± 0.048	−11	49 ± 0.62	48 ± 1.02	−2	0.72 ± 0.013	0.71 ± 0.028	−1
10	1.91 ± 0.034	1.60 ± 0.008	−16	46 ± 0.74	43 ± 0.32	7	0.60 ± 0.016	0.53 ± 0.020	−12
11	1.86 ± 0.055	1.62 ± 0.026	−13	49 ± 1.14	39 ± 0.89	−20	0.75 ± 0.005	0.73 ± 0.014	−3
Average	1.94 ± 0.025	1.60 ± 0.023	−18	44.62 ± 0.872	39.41 ± 0.655	−12	0.77 ± 0.013	0.70 ± 0.012	−9
LSD 5%	0.215	0.137	–	3.81	3.48	–	0.137	0.042	–
F-ratio	7.40	17.84	–	21.52	29.35	–	3.97	3.01	–
p-Value	0.000	0.000	–	0.000	0.000	–	0.000	0.003	–

TABLE 4B The mean comparisons of stem weight (SW), spike straw remobilization (SSR), and stem remobilization (SR) traits in various genotypes (Factor A) in irrigation (I) and rainfed (R) conditions in second year of experiment.

Genotype	SW (g)			SSR (g)			SR (g)		
	(I)	(R)	Change %	(I)	(R)	Change %	(I)	(R)	Change %
Pishgam	1.69 ± 0.079	1.49 ± 0.041	−12	0.64 ± 0.030	0.51 ± 0.029	20	0.82 ± 0.043	1.12 ± 0.054	37
Baharan	1.73 ± 0.057	1.50 ± 0.048	−13	0.03 ± 0.003	0.56 ± 0.026	143	0.77 ± 0.045	0.89 ± 0.036	16
3	2.39 ± 0.096	2.14 ± 0.088	−10	0.62 ± 0.057	0.67 ± 0.020	8	0.41 ± 0.030	1.18 ± 0.059	188
4	1.92 ± 0.053	1.29 ± 0.054	−33	0.03 ± 0.003	0.04 ± 0.003	33	0.54 ± 0.039	0.43 ± 0.046	20
5	1.84 ± 0.040	1.41 ± 0.046	−23	0.32 ± 0.025	0.72 ± 0.044	125	0.51 ± 0.032	0.79 ± 0.033	55
6	1.68 ± 0.034	1.3 ± 0.026	−23	0.15 ± 0.010	0.37 ± 0.042	147	0.79 ± 0.091	0.51 ± 0.029	35
7	2.18 ± 0.046	1.59 ± 0.036	−27	0.36 ± 0.018	0.03 ± 0.005	92	0.66 ± 0.078	0.49 ± 0.031	26
8	1.64 ± 0.062	1.36 ± 0.025	−17	0.51 ± 0.015	0.18 ± 0.025	65	0.63 ± 0.080	0.60 ± 0.027	5
9	2.09 ± 0.072	1.93 ± 0.056	−8	0.62 ± 0.025	0.73 ± 0.090	18	0.85 ± 0.033	0.81 ± 0.052	5
10	1.68 ± 0.064	1.42 ± 0.033	−15	0.46 ± 0.015	0.57 ± 0.078	24	0.41 ± 0.030	1.04 ± 0.056	154
11	1.84 ± 0.046	1.5 ± 0.051	−18	0.43 ± 0.019	0.41 ± 0.051	5	0.49 ± 0.065	1.10 ± 0.073	125
Average	1.88 ± 0.026	1.54 ± 0.025	−18	0.38 ± 0.020	0.43 ± 0.024	13	0.63 ± 0.021	0.81 ± 0.026	29
LSD 5%	0.358	0.192	–	0.216	0.238	–	0.193	0.216	–
F-ratio	4.01	16.78	–	3.91	3.11	–	3.25	3.94	–
p-Value	0.004	0.000	–	0.004	0.005	–	0.001	0.001	–

LSD, least significant difference.

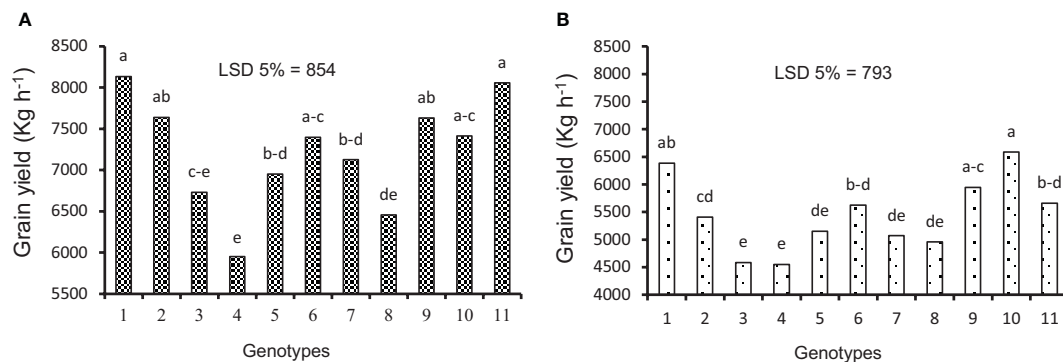


FIGURE 2

Average grain yield under (A) irrigation and (B) rainfed experiments in the second year. Means followed by the same letter within a column are not significantly different at the 5% probability level according to the least significant difference test.

(data not shown). The SSW decreased in RFL and RAL treatments (reduction of source strength) while increasing in RHS treatment (reduction of sink strength) under irrigation and rainfed conditions (Figures 8A, B). The remobilization values from spike straw to grains in check (without S-S manipulation) were 0.38 and 0.43 g/

spike in irrigation and rainfed conditions, respectively, equivalent to 20% and 27% of grain weight (Table 4). SSW decreased with RFL, and this reduction was more intense in RAL. This reduction means that the increase in remobilization from spike straw to grains was 0.04 and 0.05 g/spike in RFL under irrigation and rainfed

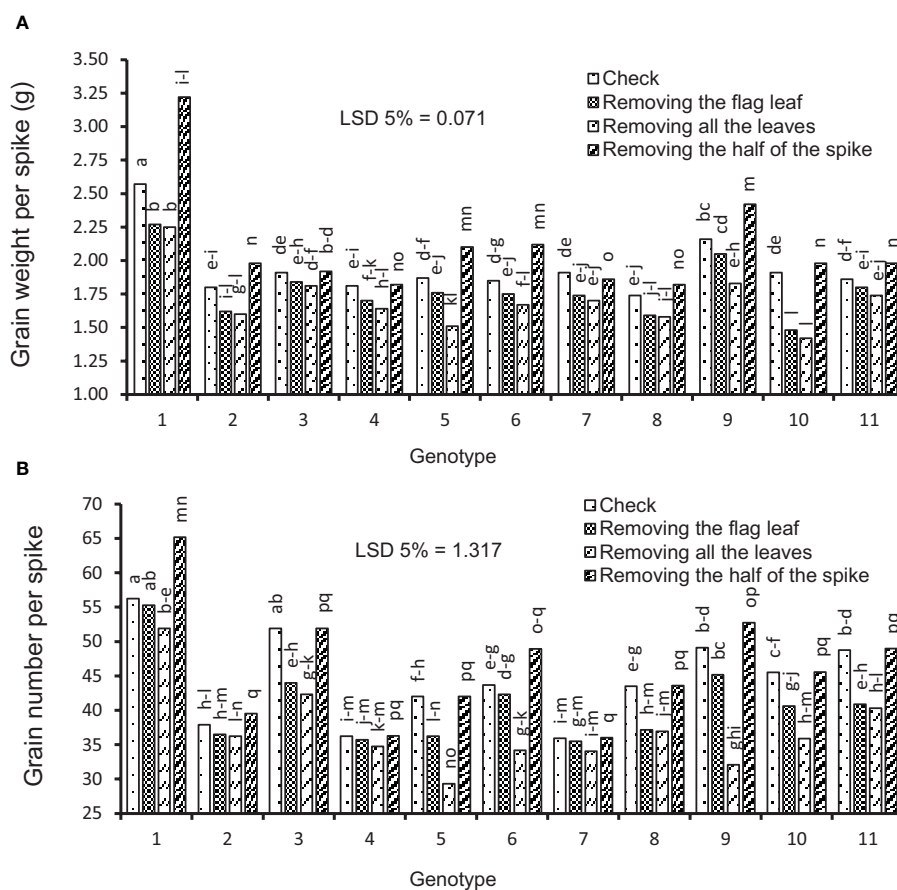


FIGURE 3

Mean comparison (under irrigation conditions) for interactions of genotypes x source-sink manipulation treatments including i) check, ii) removal of flag leaf, iii) removal of all leaves, and iv) removal of upper half of the spikes. (A) Average of grain weight per spike and (B) average of grain number per spike. Means followed by the same letter within a column are not significantly different at the 5% probability level according to the least significant difference test.

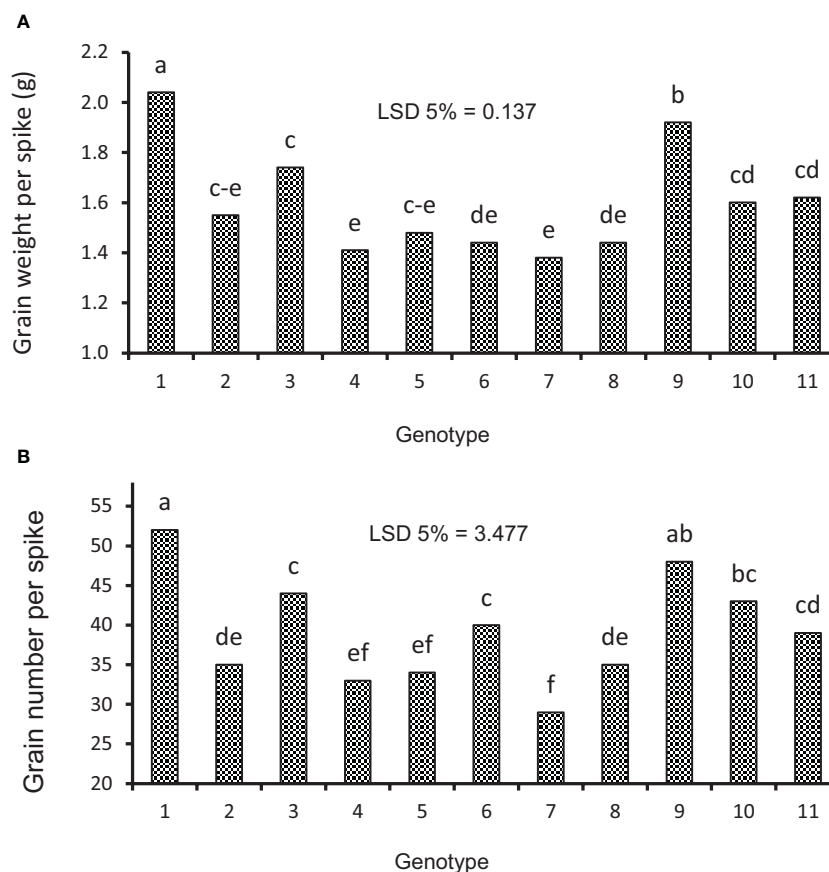


FIGURE 4

Mean comparison of genotypes under rainfed conditions: (A) average of grain weight per spike and (B) average of grain number per spike. Means followed by the same letter within a column are not significantly different at the 5% probability level according to the least significant difference test.

conditions, respectively, and 0.08 and 0.09 g/spike in RAL, respectively (Table 9). The role of this increase in remobilization under RFL was 2% and 2.5% in grain yield per spike in irrigation and rainfed conditions, respectively, and in RAL, it was 4% and 6%, respectively. Removing a part of the spike and reducing sink size reduced the need for photosynthetic materials and remobilization value from the spike straw to the grains. The increase in the GWS under the influence of RHS was 0.17 and 0.16 g/spike in irrigation and rainfed conditions, respectively (Table 6).

3.6 The compensatory role of vegetative organs in grain filling

In our study, under irrigation conditions, by RFL treatment, GWS, SW, and SSW decreased by 0.16, 0.07, and 0.04 g per plant, respectively (Table 6), equivalent to 0.27 g per plant of photosynthesis reduction. The decrease in the SW and SSW by 0.11 g per plant means an increase in the remobilization from the stem and spike straw to the grains by 0.11 g per plant, and although the photosynthesis decreased by 0.27 g per plant, grain weight decreased by only 0.16 g. Therefore, the increase in remobilization from the stem and spike straw was compensated by 41% of the decrease in photosynthesis caused by RFL in irrigation conditions.

In rainfed conditions, due to the reduction of 0.11, 0.08, and 0.04 g per plant in the GWS, SW, and SSW, respectively, the role of remobilization from vegetative organs in compensating for the decrease in photosynthesis was equal to 52%. Also, under irrigation conditions, in RAL treatment, GWS, SW, and SSW decreased by 0.24, 0.19, and 0.08 g per plant, respectively, which was equivalent to 0.51 g per plant, reducing photosynthesis. The increase in remobilization from the stem and spike straw to the amount of 0.27 g caused the grain weight to decrease by only 0.24 g. Therefore, remobilization equivalent to 53% photosynthesis reduction due to the RAL was compensated in irrigation conditions. Hence, the compensatory effect of increasing remobilization under stress conditions due to the decrease of 0.17, 0.12, and 0.09 g per plant in GWS, SW, and SSW, respectively, was 56% (Table 6). Furthermore, under irrigation conditions, by RHS, grain weight in the remaining half of the spike should have been halved and reduced from 1.94 g to 0.97 g, but it reached 1.06 g. This means that 0.09 g of material was stored. However, SW and SSW in this treatment increased by 0.51 g, and GWS, SW, and SSW increased by 0.60 g per plant. In rainfed conditions, the grain weight in the half spike in the intact plant was 0.8 g, but by RHS, the GWS in the remaining half of the spike reached 0.89 g. Also, SW and SSW increased by 0.48 g per plant, while GWS, SW, and SSW increased by 0.57 g per plant. Therefore,

TABLE 5 Correlation coefficients grain yield (kg/ha) (GY), thousand-grain weight (TGW), spike number per m² (SN), grain weight per spike (GWS), grain number per spike (GNS), spike straw remobilization (SSR), stem remobilization (SR), source limitation (SL), flag leaf removal effect (FLRE), all leaves removal effect (ALRE), chlorophyll *a* (Cha), chlorophyll *b* (Chb), total chlorophyll (TCh), and carotenoids (CA) of wheat in irrigation (down side) and rainfed (up side) conditions.

Traits	GY	TGW	SN	GWS	GNS	SSR	SR	SL	FLRE	ALRE	Cha	Chb	TCh	CA
GY	1	−0.534	0.400 ^{ns}	0.547 ^{ns}	0.637*	0.404 ^{ns}	0.437	0.702*	−0.457 ^{ns}	−0.042 ^{ns}	0.626*	0.638*	0.635*	0.545 ^{ns}
TGW	−0.370 ^{ns}	1	−0.128 ^{ns}	−0.616*	−0.851**	−0.583 ^{ns}	−0.455	−0.582 ^{ns}	0.024 ^{ns}	0.074 ^{ns}	−0.232 ^{ns}	−0.363 ^{ns}	−0.290 ^{ns}	−0.207 ^{ns}
SN	−0.042 ^{ns}	0.369 ^{ns}	1	0.031 ^{ns}	0.083 ^{ns}	0.110 ^{ns}	−0.076	0.335 ^{ns}	−0.189 ^{ns}	0.009 ^{ns}	0.410 ^{ns}	0.166 ^{ns}	0.304 ^{ns}	0.360 ^{ns}
GWS	0.560 ^{ns}	−0.367 ^{ns}	−0.398 ^{ns}	1	0.924**	0.605*	0.699*	0.632*	−0.027 ^{ns}	−0.107 ^{ns}	0.327 ^{ns}	0.370 ^{ns}	0.346 ^{ns}	0.394 ^{ns}
GNS	0.518 ^{ns}	−0.681*	−0.700*	0.671*	1	0.607*	0.657*	0.692*	−0.098 ^{ns}	−0.123 ^{ns}	0.307 ^{ns}	0.415 ^{ns}	0.350 ^{ns}	0.321 ^{ns}
SSR	0.299 ^{ns}	−0.370 ^{ns}	−0.834**	0.564 ^{ns}	0.804**	1	0.698*	0.329 ^{ns}	−0.044 ^{ns}	0.151 ^{ns}	−0.014 ^{ns}	−0.010 ^{ns}	−0.016 ^{ns}	−0.006 ^{ns}
SR	0.405 ^{ns}	−0.202 ^{ns}	0.163 ^{ns}	0.465 ^{ns}	0.037 ^{ns}	−0.062 ^{ns}	1	0.301 ^{ns}	0.121 ^{ns}	0.373 ^{ns}	0.079 ^{ns}	0.294 ^{ns}	0.217 ^{ns}	0.136 ^{ns}
SL	0.647*	−0.410 ^{ns}	−0.026 ^{ns}	0.658*	0.479 ^{ns}	0.115 ^{ns}	0.684*	1	−0.338 ^{ns}	−0.440 ^{ns}	0.581 ^{ns}	0.584 ^{ns}	0.559 ^{ns}	0.579 ^{ns}
FLRE	0.152 ^{ns}	−0.251 ^{ns}	0.063 ^{ns}	0.258 ^{ns}	0.074 ^{ns}	0.162 ^{ns}	−0.174 ^{ns}	−0.034 ^{ns}	1	0.566 ^{ns}	−0.290 ^{ns}	−0.207 ^{ns}	−0.177 ^{ns}	−0.118 ^{ns}
ALRE	0.091 ^{ns}	−0.037 ^{ns}	0.004 ^{ns}	0.161 ^{ns}	−0.041 ^{ns}	0.103 ^{ns}	−0.102 ^{ns}	0.053 ^{ns}	0.728*	1	−0.154 ^{ns}	−0.034 ^{ns}	−0.042 ^{ns}	−0.176 ^{ns}
Cha	0.665*	−0.638*	−0.083 ^{ns}	0.414 ^{ns}	0.359 ^{ns}	0.174 ^{ns}	0.464 ^{ns}	0.482 ^{ns}	0.255 ^{ns}	−0.078 ^{ns}	1	0.908**	0.968**	0.962**
Chb	0.595 ^{ns}	−0.732*	−0.166 ^{ns}	0.598 ^{ns}	0.468 ^{ns}	0.179 ^{ns}	0.458 ^{ns}	0.648*	0.374 ^{ns}	0.105 ^{ns}	0.905**	1	0.973**	0.871**
TCh	0.645*	−0.700*	−0.125 ^{ns}	0.515 ^{ns}	0.420 ^{ns}	0.181 ^{ns}	0.474 ^{ns}	0.576 ^{ns}	0.321 ^{ns}	0.011 ^{ns}	0.978**	0.974**	1	0.950**
CA	0.657*	−0.613*	−0.035 ^{ns}	0.432 ^{ns}	0.315 ^{ns}	0.114 ^{ns}	0.530 ^{ns}	0.521 ^{ns}	0.252 ^{ns}	−0.089 ^{ns}	0.992**	0.916**	0.979**	1

ns, not significant; * $p \leq 0.05$; ** $p \leq 0.01$.

TABLE 6A The mean comparisons of grain weight per spike (GWS), grain number per spike (GNS), spike straw weight (SSW) traits under check (CH), removal of flag leaf (RFL), removal of all leaves (RAL), and removal of the upper half of the spike (RHS) treatments in irrigation (I) and rainfed (R) conditions.

Treatment	GWS (g)			GNS (n)			SSW (g)		
	(I)	(R)	Change %	(I)	(R)	Change %	(I)	(R)	Change %
CH	1.94 ± 0.049	1.60 ± 0.045	−18	44.62 ± 1.28	39.41 ± 1.31	−12	0.77 ± 0.025	0.70 ± 0.024	−11
RFL	1.782 ± 0.044	1.495 ± 0.040	−16	40.85 ± 1.09	38.23 ± 1.28	−6	0.73 ± 0.021	0.66 ± 0.021	−10
Change %	−8	−7	−	−8	−3	−	−5	−6	−
RAL	1.705 ± 0.047	1.425 ± 0.034	−17	37.09 ± 0.95	36.01 ± 1.23	−3	0.69 ± 0.021	0.61 ± 0.020	−12
Change %	−12	−11	−	−17	−9	−	−10	−13	−
RHS	2.11 ± 0.041	1.77 ± 0.031	−16	46.44 ± 0.73	41.87 ± 0.77	−10	1.03 ± 0.019	0.90 ± 0.010	−13
Change %	9	11	−	4	6	−	34	29	−
LSD 5%	0.071	0.056	−	1.317	2.38	−	0.134	0.126	−
F-ratio	153.32	147.23	−	411.93	373.12	−	28.12	21.62	−
p-Value	0.000	0.000	−	0.000	0.000	−	0.000	0.000	−

TABLE 6B The mean comparisons of stem weight (SW), spike straw remobilization (SSR) and stem remobilization (SR) traits under check (CH), removal of flag leaf (RFL), removal of all leaves (RAL), and removal of the upper half of the spike (RHS) treatments in irrigation (I) and rainfed (R) conditions.

Treatment	SW (g)			SSR (g)			SR (g)		
	(I)	(R)	Change %	(I)	(R)	Change %	(I)	(R)	Change %
CH	1.88 ± 0.051	1.54 ± 0.051	−18	0.38 ± 0.040	0.43 ± 0.049	10	0.63 ± 0.042	0.81 ± 0.052	29
RFL	1.81 ± 0.070	1.46 ± 0.048	−19	0.42 ± 0.042	0.54 ± 0.021	29	0.68 ± 0.039	0.89 ± 0.062	31
Change %	−4	−5	−	11	17.39	−	8	8.99	−
RAL	1.69 ± 0.050	1.42 ± 0.045	−16	0.55 ± 0.048	0.76 ± 0.023	38	0.76 ± 0.037	0.94 ± 0.062	24
Change %	−10	−8	−	45	77	−	21	16	−
RHS	2.30 ± 0.093	1.82 ± 0.077	−21	0.048 ± 0.003	0.18 ± 0.012	275	0.067 ± 0.015	0.21 ± 0.011	213
Change %	22	18	−	−87	−58	−	−89	−74	−
LSD 5%	0.126	0.295	−	0.068	0.093	−	0.076	0.098	−
F-ratio	43.29	33.14	−	12.69	16.87	−	10.24	12.87	−
p-Value	0.000	0.000	−	0.000	0.000	−	0.000	0.000	−

To make uniform observations in the data and figures, the values of GWS, GNS, and SSW in grams have been doubled in RHS treatment.

LSD, least significant difference.

the production of photosynthetic materials decreased by only 0.23 g per plant, equivalent to 29% of the weight of the half spike (demand reduction). Results in this study showed a significant positive relationship between stem remobilization with the GWS ($r = 0.699^*$) and the GNS ($r = 0.657^*$) as well as a significant positive relationship between the spike straw remobilization with the GWS ($r = 0.605^*$) and the GNS ($r = 0.607^*$) in rainfed conditions (Table 5). Moreover, a significant positive relationship ($r = 0.684^*$) was observed between the remobilization of the stem under irrigation conditions and source limitation (Table 5).

3.7 Remobilization of water-soluble carbohydrates in the stem

The obtained results in this study revealed the substantial genetic variations of the WSC remobilization and efficiency from the stems (Table 10). This is consistent with the findings of other studies (Ehdaie et al., 2006a; Ehdaie et al., 2006b; Vosoghi Rad et al., 2022), which, accordingly, corroborate the manipulation of this trait in wheat breeding programs. Accordingly, depending on the cultivars and the environmental conditions (irrigation and

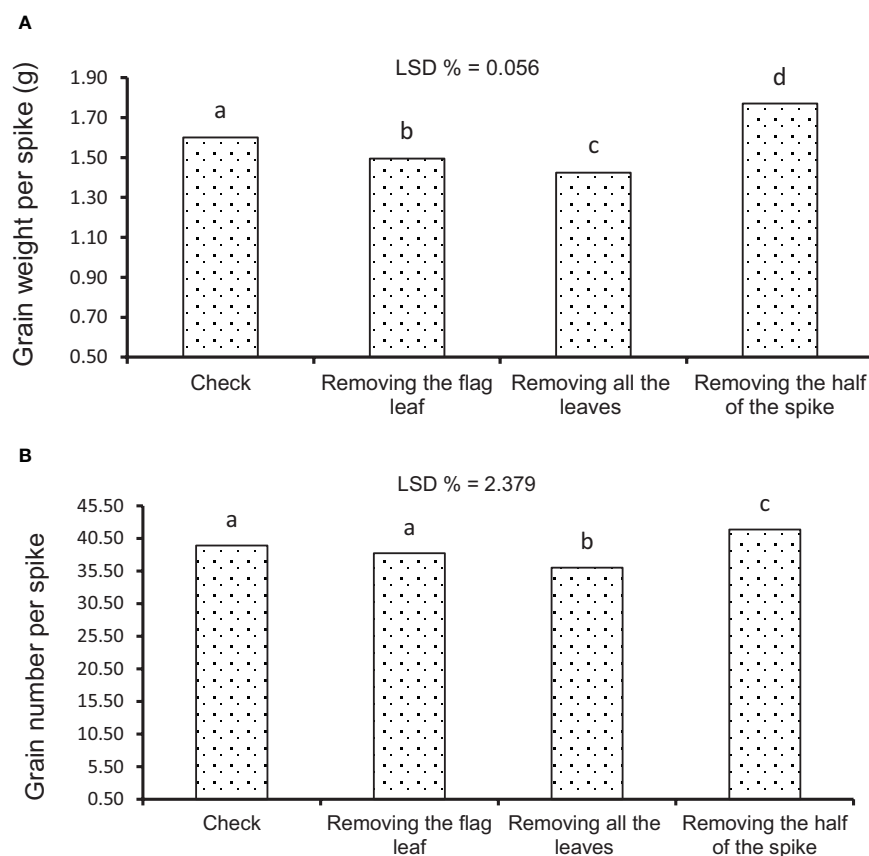


FIGURE 5

Mean comparison of source-sink treatments under rainfed conditions. (A) Average of grain weight per spike. (B) Average of grain number per spike. Means followed by the same letter within a column are not significantly different at the 5% probability level according to the least significant difference test.

rainfed), the amount of WSC content at 10 days after anthesis was estimated at 61 to 117 and at maturity at 23 to 58 g/m². Also, the average values of WSC remobilization in irrigation and rainfed conditions were estimated at 52.85 and 47.66 g/m², respectively. The average WSC remobilization in all genotypes in irrigation and rainfed conditions was 52.85 and 47.66 g/m², respectively. Furthermore, the average remobilization efficiency (%) in irrigation and rainfed conditions was 53.10% and 59.18%, respectively, which increased by 11.45% in rainfed conditions (Table 10). Also, the contribution of WSC remobilization in grain yield (%) in rainfed conditions has increased by 19% compared to that in irrigation conditions. Results show the effect and importance of WSC remobilization in drought stress conditions. No strong correlation was observed between the above traits and grain yield.

3.8 Chlorophyll

The results showed that in both conditions, the effect of genotype on chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoids was significant (data not shown), and there was a significant difference between the genotypes (Table 11). Also, drought stress caused a decrease in chlorophyll contents so that the values of chlorophyll *a*, chlorophyll *b*, total, and carotenoids in

all genotypes decreased to 20.7%, 24.10%, 21.02%, and 16%, respectively (Table 11; Figures 9A–D). In addition, the values of chlorophyll and carotenoids had a significant positive correlation with grain yield (ha⁻¹) in both irrigation and rainfed conditions. In this regard, genotypes 1, 2, 6, 9, and 10 with the highest grain yield (ha⁻¹) had the highest amounts of chlorophyll. Also, the values of chlorophyll *b* showed a significant positive relationship ($r = 0.648^*$) with source limitation in irrigation conditions (Table 5).

4 Discussion

4.1 Relationships between source-sink affected by RFL, RAL, and RHS treatments

As expected, grain yield was closely related to GNS. The results showed that lines with more GNS had higher GWS and higher grain yield (per ha⁻¹). Grain yield is strongly related to the number of grains harvested at physiological maturity (Peltonen-Sainio et al., 2007; Fischer, 2008). Although grain number is the dominant component of grain yield determination, it is evident that for any given number, there is a wide range of achievable yield due to variations in grain weight (Slafer et al., 2014). Drought stress caused a significant decrease in GNS (12%) and GWS (18%) compared to

TABLE 7 Average grain weight per spike (GWS) in check (CH), removal of the upper half of the spikes (RHS), removal of flag leaf (RFL), removal of all leaves (RAL), and source limitation (SL) based on average grain weight per spike (GWS) in RHS treatment compared to check in irrigation (I) and rainfed (R) experiments.

Genotype	Average GWS in CH (g)		Average GWS in RHS (g)		Source limitation (%)		Average grain weight in RFL (g)		Effect of RFL (%)		Average grain weight in RAL (g)		Effect of RAL (%)	
Site	(I)	(R)	(I)	(R)	(I)	(R)	(I)	(R)	(I)	(R)	(I)	(R)	(I)	(R)
Pishgam	0.046	0.039	0.057	0.051	23.91	30.77	0.040	0.038	13.04	2.56	0.040	0.037	13.04	5.13
Baharan	0.047	0.044	0.052	0.046	10.64	4.54	0.043	0.042	8.51	4.54	0.042	0.037	10.64	15.91
3	0.037	0.039	0.037	0.040	0.52	2.56	0.035	0.035	5.40	10.26	0.035	0.034	5.40	12.82
4	0.050	0.043	0.050	0.046	0.55	6.98	0.047	0.037	6.01	13.95	0.045	0.037	10.01	13.95
5	0.045	0.044	0.050	0.050	11.11	13.64	0.042	0.041	6.66	6.82	0.036	0.040	20.01	9.09
6	0.042	0.036	0.049	0.041	16.66	13.89	0.040	0.035	4.76	2.78	0.038	0.034	9.52	5.55
7	0.053	0.047	0.053	0.048	0.01	2.13	0.048	0.046	9.43	2.13	0.047	0.044	11.32	6.38
8	0.040	0.041	0.042	0.043	5.01	4.88	0.037	0.039	7.50	4.88	0.036	0.037	10.02	9.76
9	0.044	0.040	0.049	0.045	11.36	12.50	0.042	0.037	4.54	7.50	0.037	0.036	15.91	10.01
10	0.042	0.037	0.043	0.042	2.38	13.51	0.033	0.036	21.43	2.70	0.031	0.031	26.19	16.22
11	0.038	0.041	0.041	0.044	7.89	7.32	0.037	0.035	2.63	14.63	0.036	0.034	5.26	17.07
Average	0.044	0.041	0.048	0.045	8.18	10.25	0.040	0.038	8.17	6.61	0.038	0.036	12.48	11.08

To make uniform observations in the data and figures, the values of grain weight per spike (GWS) in grams have been doubled in RHS treatment.

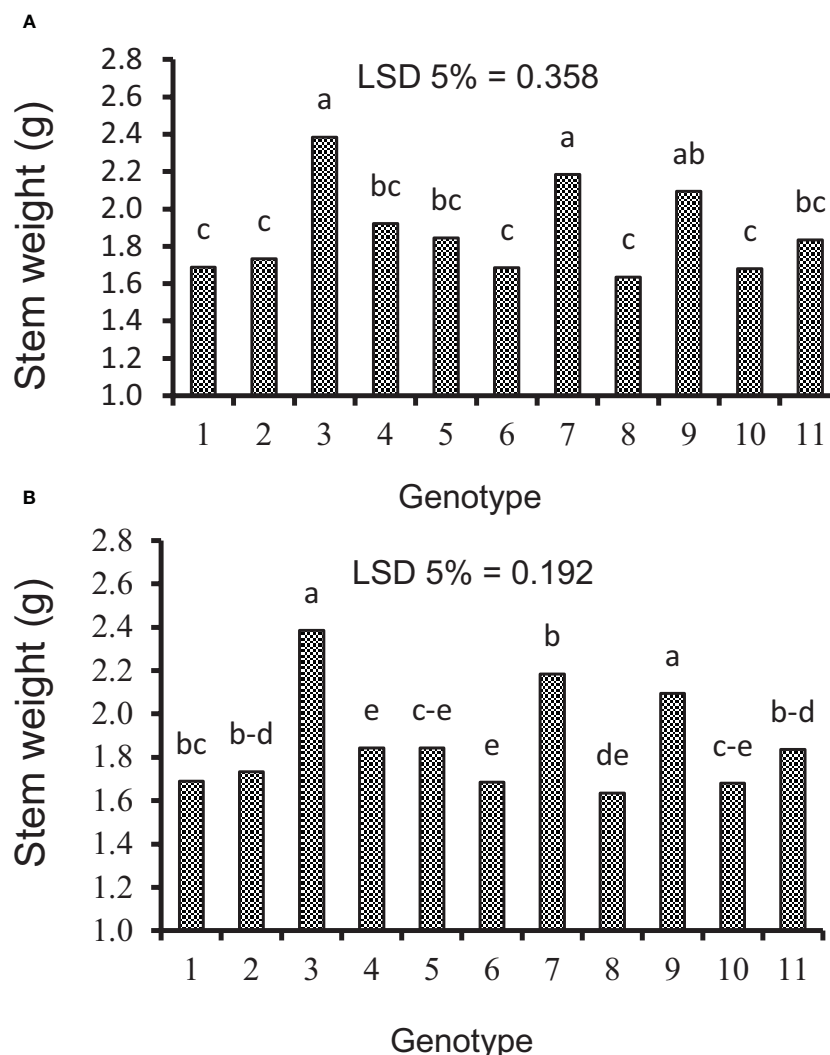


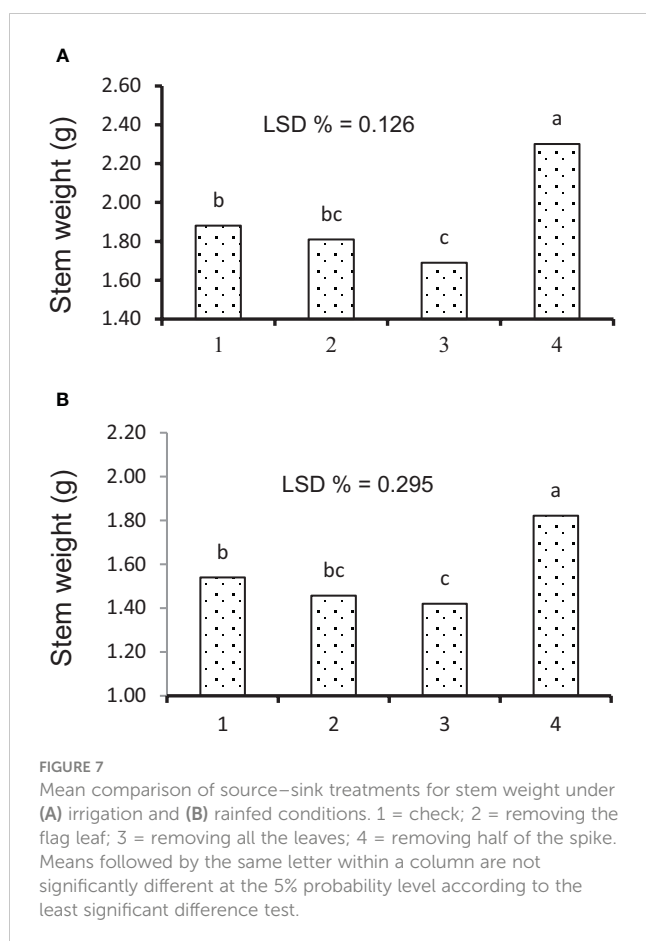
FIGURE 6

Mean comparison of genotypes for stem weight under (A) irrigation and (B) rainfed conditions. Means followed by the same letter within a column are not significantly different at the 5% probability level according to the least significant difference test.

irrigation conditions (Table 4). The grain weight of Ghods wheat cultivars significantly decreased under drought stress (Ahmadi et al., 2009). In RFL, the average decrease in GNS was 8% and 3% in rainfed and irrigation conditions, respectively, and in RAL, it was 17% and 9%, respectively (Table 6). Considering that leaf removal treatments were applied 2 weeks after anthesis, and at this stage, pollination and grain number were not affected, it can be said that the decrease in grain number was due to the abortion of grains or the formation of tiny grains that were not considered in the counting process. It has been reported that the removal of all leaves partially reduced the grain number of wheat by 3% to 6% (Zhenlin et al., 1998). In a study with 20 cultivars and lines of wheat, it was shown that GNS was significantly reduced by the removal of all leaves after pollination (Alam et al., 2008). Also, the RHS in some lines (lines 1, 6, and 9) increased the number of the grains in the remaining half of the spike by 4% and 6% in irrigation and rainfed, respectively (Table 6), which seems to be due to competition

reduction between grains in absorbing assimilation and better growth of grains and prevention of grain abortion.

The contribution rate of flag leaves to daily photosynthetic products varies from 50% to 60% (Towfiq et al., 2015), while its defoliation generated grain yield losses of 18% to 30% (Ma et al., 2021). It has been reported that different intensities of leaf removal in the beginning stage of sink capacity formation cause a significant reduction in grain weight in different wheat cultivars (Bijanazadeh and Emam, 2010), and through the reduction in photosynthesis, it causes a decrease in grain yield (Albacete et al., 2014). Singh and Singh 1992 showed that source restriction reduced the 30% to 40% yield of wheat cultivars. Bijanazadeh and Emam (2010) announced that in the Shiraz cultivar, defoliation of all leaves decreased the main shoot yield by 40.75%. This demonstrated that the Shiraz cultivar was sensitive to source restriction under well-watered conditions. Generally, genetic diversity was observed among wheat cultivars when they were imposed on source restriction and drought stress.



It has been stated that the removal of all leaves and flag leaves caused a 28% and 17% decrease in grain weight per spike, respectively (Alam et al., 2008), which indicates the limitation of the source strength. In this study, it seems that in lines 1 and 10 under irrigation conditions, which had more yield and more oversized sink, under the influence of RFL, the limitation of photosynthetic materials in them increased, and yield reduction has been more severe. In addition, lines 3 and 4, which had lower grain yield potential, showed less sensitivity to source reduction probably due to relative sink limitation (Figures 3A, B; Tables 4, 6, 11). Therefore, the flag leaf is decisive in the S-S relationship. Researchers observed a 7%–9% decrease in grain weight, 10.7% in grain yield, and 11.1% in GNS during an experiment by removing the flag leaf after spike formation (Sharma et al., 2003). In the RAL, the average decrease in GWS compared to check was 12% and 11% in irrigation and rainfed conditions, respectively, and genotypes 3 and 11 in irrigation conditions and genotypes 6 and 7 in rainfed showed the least reaction to the RAL, which was probably due to the low source limitation and the relative sink limitation in them (Tables 6, 7). Therefore, considering that source reduction in most of the studied lines caused a significant reduction in grain weight per spike, these genotypes have some source limitations (Table 7). The role of RAL in the reduction of GWS was stronger than that of RFL which was calculated through Equation 4. However, no significant difference was observed in most of the genotypes, which shows the importance and role of the flag leaf in

photosynthesis and the production of assimilates (Table 7). Investigating S-S relationships in more than 150 new bread wheat genotypes showed that all genotypes have some source limitation in sink capacity levels (Alonso et al., 2018). In general, the results showed that the leaves had a lesser role in grain filling in rainfed conditions, and grain filling under drought stress is more dependent on the remobilization of photosynthetic materials. Moreover, results showed an increase in GWS in the RHS treatment, which is interpreted as source limitation. Of course, these results indicate the limitation of the sink because, with the doubling of available photosynthetic materials under RHS treatment, the grain weight in the remaining half of the spike increased by only 10% on average. There is a significant positive relationship between source limitation and grain yield (kg/ha) in both irrigation ($r = 0.647^*$) and rainfed ($r = 0.702^*$) conditions, according to the results of Modhej (2011) (Table 5).

Hence, it can be said that in genotypes with higher grain yield (kg/ha), more GNS, or higher GWS (due to the more oversized sink), photosynthetic material limitation is more severe in them, and as a result, the reduction of grain yield in these genotypes under defoliation treatments will be higher. All of these genotypes are included in the category of limited source (Table 7). In this regard, it has been reported that the genotypes with a more oversized sink (grain number) will have more source limitations due to the increased competition of the sinks to receive photosynthetic materials (Satorre and Slafer, 2000). Some researchers have stated that due to the role of sources during the grain-filling period, genotypes that have less source limitation under normal and drought stress conditions have a higher genetic potential for grain yield (Janmohammadi et al., 2010). However, despite this issue, the investigated genotypes in this experiment, which are from the advanced lines resulting from the breeding experiments of Icarda and Simit, had a relative source limitation due to favorable sink potential. The studies conducted on improved wheat cultivars for different regions show that the source limitation has increased during the improvement programs in the direction of more grains and more yield in wheat and that the newly modified varieties of wheat have source limitations due to the increase in sink strength and related traits such as more oversized spikes and more GNS (Koshkin and Tararina, 2003; Alonso et al., 2018; Kuzay et al., 2019).

In recent research, the understanding of the genetic basis of source-related traits has been emphasized because the increase of spikelets in the spike can lead to more yield only when the source is adapted to the rise in the sink at the same time (Kuzay et al., 2019). It has been reported that defoliation reduces both the traits of grain growth rate and grain weight of cultivars. However, the relative reduction for sensitive cultivars to spike removal (limited source cultivars) is greater than that for insensitive cultivars (Abdoli and Saeidi, 2013). Complementary crosses between genotypes with high sink capacity and those with high source capacity resulted in progeny with substantial yield improvement (Reynolds et al., 2017), suggesting the co-limitation of S-S on yield. It has been suggested that selecting crossing partners based on physiological traits is a promising strategy to achieve higher crop productivity through breeding, which is facilitated by the increasingly automated

TABLE 8 Stem weight (SW) in check (CH), removal of flag leaf (RFL), removal of all leaves (RAL) and removal of the upper half of the spike (RHS) treatments, stem remobilization increase affected by RFL and RAL treatments, and stem remobilization decrease affected by RHS in grams (g) in irrigation (I) and rainfed (R) experiments.

Genotype	SW in CH treatment (g)		SW in RFL treatment (g)		SW in RAL treatment (g)		SW in RHS treatment (g)		Stem remobilization increase affected by RFL treatment (g)		Stem remobilization increase affected by RAL treatment (g)		Stem remobilization decrease affected by RHS treatment (g)	
Site	(I)	(R)	(I)	(R)	(I)	(R)	(I)	(R)	(I)	(R)	(I)	(R)	(I)	(R)
Pishgam	1.69	1.49	1.67	1.44	1.6	1.42	1.98	1.88	0.02	0.05	0.09	0.07	−0.29	−0.39
Baharan	1.73	1.5	1.66	1.37	1.59	1.27	2.07	1.9	0.07	0.13	0.14	0.23	−0.34	−0.4
3	2.39	2.14	2.3	2.1	2.19	2.07	2.81	2.39	0.09	0.04	0.20	0.07	−0.42	−0.25
4	1.92	1.29	1.84	1.21	1.71	1.28	2.13	1.48	0.08	0.08	0.21	0.01	−0.21	−0.19
5	1.84	1.41	1.8	1.34	1.63	1.31	2.2	1.67	0.04	0.07	0.21	0.10	−0.36	−0.26
6	1.68	1.3	1.52	1.22	1.44	1.3	2.19	1.44	0.16	0.09	0.24	0.01	−0.51	−0.13
7	2.18	1.59	2.12	1.5	1.93	1.41	2.9	1.82	0.06	0.09	0.25	0.18	−0.72	−0.23
8	1.64	1.36	1.55	1.35	1.49	1.25	1.9	1.54	0.09	0.01	0.15	0.11	−0.26	−0.18
9	2.09	1.93	2.05	1.75	1.78	1.66	2.69	2.61	0.04	0.18	0.31	0.27	−0.6	−0.68
10	1.68	1.42	1.61	1.4	1.59	1.27	2.1	1.62	0.07	0.02	0.09	0.15	−0.42	−0.2
11	1.84	1.5	1.81	1.41	1.67	1.36	2.33	1.71	0.03	0.09	0.17	0.14	−0.49	−0.21
Average	1.88	1.54	1.81	1.46	1.69	1.42	2.30	1.82	0.07	0.08	0.10	0.12	−0.42	−0.28

To make uniform observations in the data and figures, the values of SW in grams have been doubled in RHS treatment.

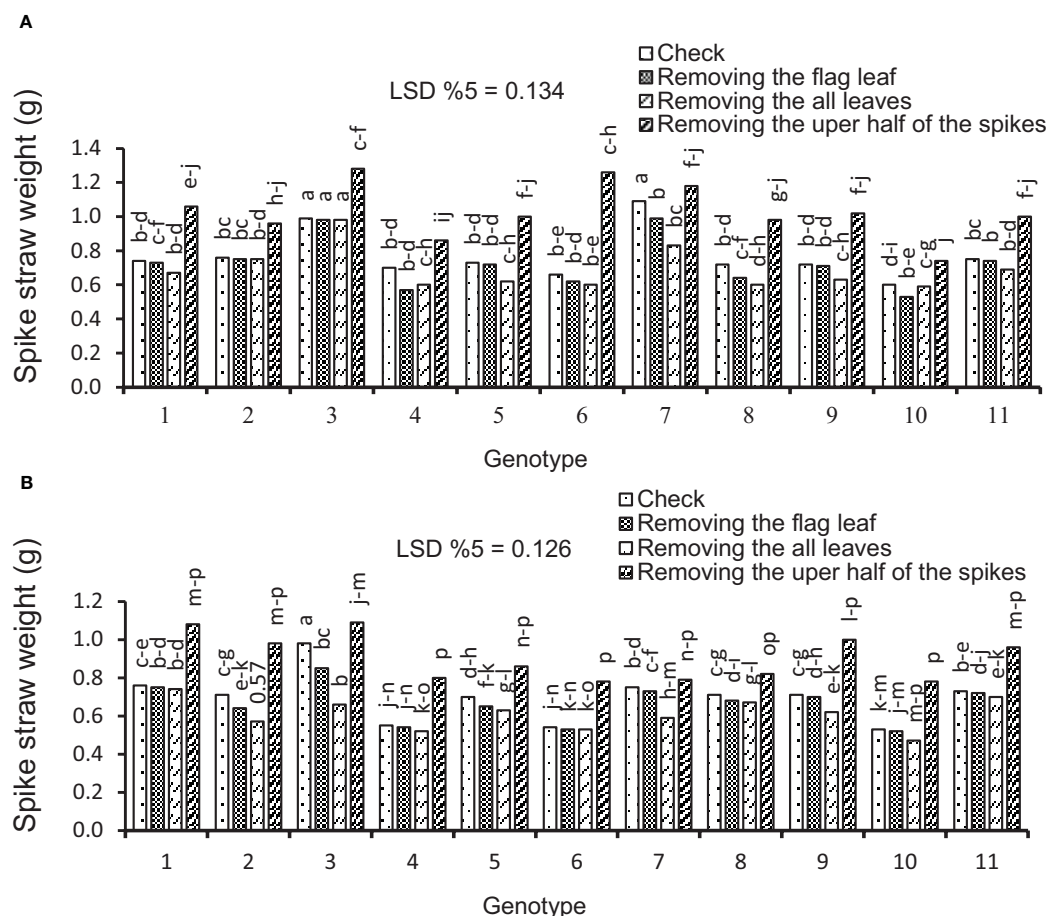


FIGURE 8

Mean comparison for interactions of genotypes $\times$ source-sink manipulation treatments including i) check, ii) removal of flag leaf, iii) removal of all leaves, and iv) removal of the upper half of the spikes. (A) Average spike straw weight under irrigation conditions. (B) Average spike straw weight under rainfed conditions. Means followed by the same letter within a column are not significantly different at the 5% probability level according to the least significant difference test.

phenotyping techniques (Reynolds et al., 2017; Furbank et al., 2019). The co-limitation of S-S implies that their breeding progress should be achieved parallelly. However, the interactions of source characteristics with the sink traits and thereby their role in the breeding progress of winter wheat are unclear, especially for the capacity of the canopy to stay green (Jagadish et al., 2015).

4.2 Remobilization of stem and spike straw under RFL, RAL, and RHS treatments and the compensatory role of vegetative organs in grain filling

In the grain-filling duration, the current photosynthesis is not enough for the grain's needs, so grains rely on the remobilization of stem storage materials. In this sense, remobilization is one of the plant's compensatory processes in facing the weakness of source strength, especially under drought stress. This situation may occur under source limitation conditions caused by defoliation. In wheat, the defoliation of the flag leaf blade increased the contribution of

assimilates to the grain from the stem and the chaff under normal conditions (Alvaro et al., 2008), and the removal of these affected the grain yields under normal or water-limiting conditions (Cruz-Aguado et al., 1999). In this study, the RFL treatment caused a significant decrease in SW compared to the check, as well as in RAL; the reduction in SW and the increase in remobilization to grains were intensified (Table 8). Leaf removal treatment increases the remobilization of non-structural carbohydrates in the stem (Noshin et al., 1996). This decrease in SW affected by reduction in photosynthesis rate stimulates compensatory mechanisms including the remobilization of storage materials from the stem, especially at higher intensities of leaf removal and in drought stress conditions. Other findings demonstrated a significant increase in the rate and efficiency of assimilate remobilization from the stem internodes under drought stress (Ma et al., 2014; Vosoghi Rad et al., 2022). Hence, more storage materials are sent from the stems to the economic sinks (grains) and, as a result, will cause a further reduction in the SW. The researchers stated that under low-to-medium conditions of source limitation, the plant resists nutrient deficiency stress by physiological mechanisms including more optimally using the reserves in the aerial

TABLE 9 Spike straw weight (SSW) in check (CH), removal of flag leaf (RFL), removal of all leaves (RAL) and removal of the upper half of the spikes (RHS) treatments, spike straw remobilization increase affected by RFL and RAL treatments, and spike straw remobilization decrease affected by RHS treatment in grams (g) in irrigation (I) and rainfed (R) experiments.

Genotype	SSW in CH treatment (g)		SSW in RFL treatment (g)		SSW in RAL treatment (g)		SSW in RHS treatment (g)		Spike straw remobilization increase affected by RFL treatment (g)		Spike straw remobilization increase affected by RAL treatment (g)		Spike straw remobilization decrease affected by RHS treatment (g)	
Site	(I)	(R)	(I)	(R)	(I)	(R)	(I)	(R)	(I)	(R)	(I)	(R)	(I)	(R)
Pishgam	0.74	0.76	0.73	0.73	0.67	0.74	1.06	1.08	0.01	0.01	0.07	0.02	-0.32	-0.32
Baharan	0.76	0.71	0.75	0.64	0.75	0.57	0.96	0.98	0.01	0.07	0.01	0.14	-0.20	-0.27
3	0.99	0.98	0.98	0.85	0.98	0.66	1.28	1.09	0.01	0.13	0.01	0.32	-0.29	-0.11
4	0.70	0.55	0.57	0.54	0.60	0.52	0.86	0.80	0.13	0.01	0.10	0.03	-0.16	-0.25
5	0.73	0.70	0.72	0.65	0.62	0.63	1.01	0.86	0.01	0.05	0.11	0.07	-0.27	-0.16
6	0.66	0.54	0.62	0.53	0.60	0.53	1.26	0.78	0.04	0.01	0.06	0.01	-0.60	-0.24
7	1.09	0.76	0.99	0.70	0.83	0.59	1.18	0.79	0.10	0.06	0.26	0.16	-0.09	-0.04
8	0.72	0.71	0.64	0.68	0.60	0.67	0.98	0.82	0.08	0.03	0.12	0.04	-0.26	-0.11
9	0.72	0.71	0.71	0.70	0.63	0.62	1.02	1.01	0.01	0.01	0.09	0.09	-0.30	-0.29
10	0.60	0.53	0.53	0.51	0.59	0.47	0.74	0.78	0.07	0.01	0.01	0.06	-0.14	-0.25
11	0.75	0.73	0.74	0.68	0.69	0.70	1.01	0.96	0.01	0.01	0.06	0.03	-0.25	-0.23
Average	0.77	0.70	0.73	0.65	0.69	0.61	1.03	0.90	0.04	0.05	0.08	0.09	-0.26	-0.21

To make uniform observations in the data and figures, the values of spike straw weight (SSW) in grams have been doubled in RHS treatment.

TABLE 10A Means of the WSC content at 10 days after anthesis and maturity (g/m²) under irrigation and rainfed conditions.

Genotype	WSC content at 10 days after anthesis (g/m ²)		WSC content at maturity (g/m ²)	
	(I)	(R)	(I)	(R)
Pishgam	109.06 ± 4.66	74.45 ± 16.79	42.78 ± 5.22	25.34 ± 5.59
Baharan	80.26 ± 4.64	93.29 ± 11.56	58.00 ± 2.07	39.07 ± 6.38
3	96.79 ± 11.08	77.51 ± 5.59	30.64 ± 1.47	42.20 ± 2.07
4	108.83 ± 5.88	76.23 ± 3.54	44.56 ± 3.75	23.83 ± 2.31
5	97.19 ± 3.79	103.87 ± 3.48	56.22 ± 2.95	33.54 ± 6.33
6	98.49 ± 12.28	76.15 ± 7.25	48.94 ± 3.64	33.89 ± 7.24
7	89.89 ± 11.26	87.82 ± 8.33	41.29 ± 8.17	50.87 ± 5.07
8	61.66 ± 1.61	71.80 ± 0.44	38.47 ± 3.23	24.36 ± 2.64
9	116.84 ± 2.32	66.17 ± 2.71	35.92 ± 1.24	26.15 ± 1.92
10	116.24 ± 1.15	70.50 ± 0.81	40.80 ± 0.97	26.01 ± 0.15
11	82.01 ± 1.58	84.73 ± 14.34	38.30 ± 3.20	32.94 ± 3.12
Average	96.12 ± 3.31	80.23 ± 2.85	43.27 ± 1.69	32.57 ± 1.84
LSD 5%	17.78	24.92	10.61	12.24
F-ratio	7.84	1.74	5.34	4.37
p-Value	0.0001	0.1393	0.0007	0.0024

TABLE 10B Means of the WSC remobilization (g/m²) of the stem, remobilization efficiency (%), and contribution of WSC remobilization in grain yield (%) under irrigation and rainfed conditions.

Genotype	WSC remobilization (g/m ²)		Remobilization efficiency (%)		Contribution of WSC remobilization in grain yield (%)	
	(I)	(R)	(I)	(R)	(I)	(R)
Pishgam	66.28 ± 8.56	49.10 ± 11.51	60.41 ± 6.08	65.58 ± 1.95	8.29 ± 1.39	7.57 ± 1.55
Baharan	22.26 ± 6.47	54.22 ± 5.70	27.00 ± 6.40	58.48 ± 2.16	2.89 ± 0.71	10.62 ± 2.40
3	66.15 ± 12.12	35.31 ± 7.66	67.27 ± 4.75	44.58 ± 6.71	9.92 ± 1.97	7.72 ± 1.75
4	64.27 ± 7.56	52.39 ± 5.13	58.72 ± 4.71	68.46 ± 4.01	11.53 ± 2.57	11.51 ± 1.03
5	40.98 ± 6.71	70.32 ± 2.85	41.75 ± 5.34	68.05 ± 5.03	6.02 ± 1.26	13.66 ± 0.41
6	49.55 ± 11.66	42.27 ± 8.66	48.87 ± 6.46	55.37 ± 9.08	6.80 ± 1.75	7.66 ± 1.84
7	48.60 ± 4.43	36.94 ± 4.68	54.87 ± 4.74	42.03 ± 2.87	6.96 ± 1.02	7.25 ± 0.71
8	23.19 ± 1.89	47.44 ± 2.97	37.77 ± 3.73	66.04 ± 3.85	3.58 ± 0.09	9.62 ± 0.86
9	80.92 ± 3.56	40.02 ± 0.84	69.19 ± 1.68	60.58 ± 1.29	10.73 ± 0.85	6.75 ± 0.17
10	75.44 ± 1.71	44.49 ± 0.85	64.89 ± 0.98	63.10 ± 0.50	10.19 ± 0.32	6.77 ± 0.28
11	43.72 ± 2.72	51.78 ± 14.14	53.35 ± 3.55	58.69 ± 8.65	5.44 ± 0.30	9.00 ± 2.21
Average	52.85 ± 3.75	47.66 ± 2.44	53.10 ± 2.52	59.18 ± 1.96	7.49 ± 0.59	8.92 ± 0.52
LSD 5%	19.18	20.96	13.52	14.35	3.07	4.36
F-ratio	9.12	1.91	8.33	3.35	7.75	2.26
p-Value	0.0001	0.1052	0.0001	0.0103	0.0001	0.05

Remobilization efficiency was calculated as (mobilized WSCs/maximum weight) × 100.

LSD, least significant difference; WSC, water-soluble carbohydrate; I, irrigation; R, rainfed.

TABLE 11A The mean comparisons of grain yield (GY), thousand-grain weight (TGW), and spike number per m² (SN) in irrigation (I) and rainfed (R) conditions in second year of experiment.

Genotype	GY (kg/ha)		TGW (g)		SN (n)	
Site	(I)	(R)	(I)	(R)	(I)	(R)
Pishgam	8,133 ± 402.53	6,382 ± 275.10	39.44 ± 0.74	34.05 ± 0.51	560 ± 24.98	559 ± 43.59
Baharan	7,639 ± 666.39	5,405 ± 730.72	43.29 ± 1.27	39.16 ± 1.04	753 ± 33.41	616 ± 62.86
3	6,729 ± 219.82	4,584 ± 110.13	41.07 ± 0.53	35.03 ± 0.75	505 ± 55.35	465 ± 29.06
4	5,949 ± 837.53	4,550 ± 116.20	44.07 ± 0.84	38.52 ± 0.69	653 ± 96.51	535 ± 39.75
5	6,950 ± 353.50	5,153 ± 221.42	46.51 ± 1.09	37.44 ± 0.95	599 ± 9.33	524 ± 28.10
6	7,397 ± 232.99	5,623 ± 222.32	38.83 ± 0.24	33.49 ± 0.40	589 ± 30.14	604 ± 10.07
7	7,125 ± 642.10	5,072 ± 136.00	46.69 ± 0.72	42.39 ± 0.31	600 ± 49.15	501 ± 51.35
8	6,457 ± 376.55	4,958 ± 187.68	41.53 ± 0.62	36.91 ± 0.90	585 ± 28.69	485 ± 12.72
9	7,632 ± 665.50	5,948 ± 267.09	40.67 ± 0.77	33.76 ± 1.20	536 ± 71.59	536 ± 40.86
10	7,412 ± 242.84	6,587 ± 216.43	39.28 ± 0.75	34.47 ± 0.53	591 ± 15.03	524 ± 33.55
11	8,058 ± 470.28	5,660 ± 234.59	42.17 ± 1.02	36.37 ± 0.58	592 ± 19.73	549 ± 28.55
Average	7,226 ± 169.34	5,447 ± 135.26	42.14 ± 0.506	36.51 ± 0.502	597 ± 15.95	536 ± 12.15
LSD 5%	854	793	1.94	2.04	116	111
F-ratio	5.33	6.26	17.37	15.81	2.66	1.48
p-Value	0.0007	0.0003	0.0001	0.0001	0.030	0.219

TABLE 11B The mean comparisons of chlorophyll *a* (Cha), chlorophyll *b* (Chb), total chlorophyll (TCh), and carotenoids (CA) in irrigation (I) and rainfed (R) conditions in second year of experiment.

Genotype	Cha (mg/g)		Chb (mg/g)		TCh (mg/g)		CA (mg/g)	
Site	(I)	(R)	(I)	(R)	(I)	(R)	(I)	(R)
Pishgam	2.05 ± 0.030	1.76 ± 0.083	1.23 ± 0.055	0.98 ± 0.161	3.28 ± 0.071	2.74 ± 0.078	0.89 ± 0.030	0.85 ± 0.014
Baharan	1.70 ± 0.027	1.45 ± 0.062	0.72 ± 0.011	0.56 ± 0.047	2.42 ± 0.039	2.01 ± 0.122	0.75 ± 0.038	0.64 ± 0.045
3	1.49 ± 0.076	0.86 ± 0.079	0.62 ± 0.083	0.32 ± 0.018	2.11 ± 0.059	1.18 ± 0.075	0.65 ± 0.015	0.38 ± 0.013
4	1.51 ± 0.025	1.22 ± 0.095	0.69 ± 0.053	0.48 ± 0.091	2.20 ± 0.079	1.69 ± 0.043	0.66 ± 0.042	0.55 ± 0.082
5	1.39 ± 0.044	1.29 ± 0.049	0.57 ± 0.061	0.51 ± 0.046	1.89 ± 0.108	1.74 ± 0.087	0.61 ± 0.086	0.57 ± 0.016
6	2.03 ± 0.055	1.35 ± 0.155	1.14 ± 0.043	0.56 ± 0.061	3.17 ± 0.104	1.91 ± 0.176	0.89 ± 0.040	0.59 ± 0.086
7	1.79 ± 0.064	1.21 ± 0.167	0.75 ± 0.061	0.43 ± 0.060	2.54 ± 0.047	1.64 ± 0.197	0.79 ± 0.047	0.55 ± 0.072
8	1.73 ± 0.102	1.63 ± 0.119	0.89 ± 0.023	0.74 ± 0.078	2.59 ± 0.050	2.47 ± 0.258	0.79 ± 0.035	0.74 ± 0.029
9	1.74 ± 0.050	1.48 ± 0.084	0.81 ± 0.030	0.59 ± 0.088	2.56 ± 0.055	2.08 ± 0.091	0.75 ± 0.025	0.70 ± 0.006
10	1.87 ± 0.063	1.40 ± 0.110	0.98 ± 0.064	0.77 ± 0.046	2.85 ± 0.086	2.17 ± 0.144	0.79 ± 0.051	0.57 ± 0.021
11	1.92 ± 0.049	1.52 ± 0.204	0.85 ± 0.053	0.81 ± 0.139	2.76 ± 0.121	2.52 ± 0.117	0.81 ± 0.029	0.77 ± 0.034
Average	1.74 ± 0.039	1.38 ± 0.050	0.84 ± 0.037	0.61 ± 0.039	2.58 ± 0.074	2.01 ± 0.084	0.76 ± 0.018	0.63 ± 0.024
LSD 5%	0.22	0.32	0.20	0.26	0.40	0.49	0.10	0.13
F-ratio	9	5.25	10.11	5.39	10.01	7.23	7.48	7.37
p-Value	0.0001	0.0008	0.0001	0.0007	0.0001	0.0001	0.0001	0.0001

LSD, least significant difference.

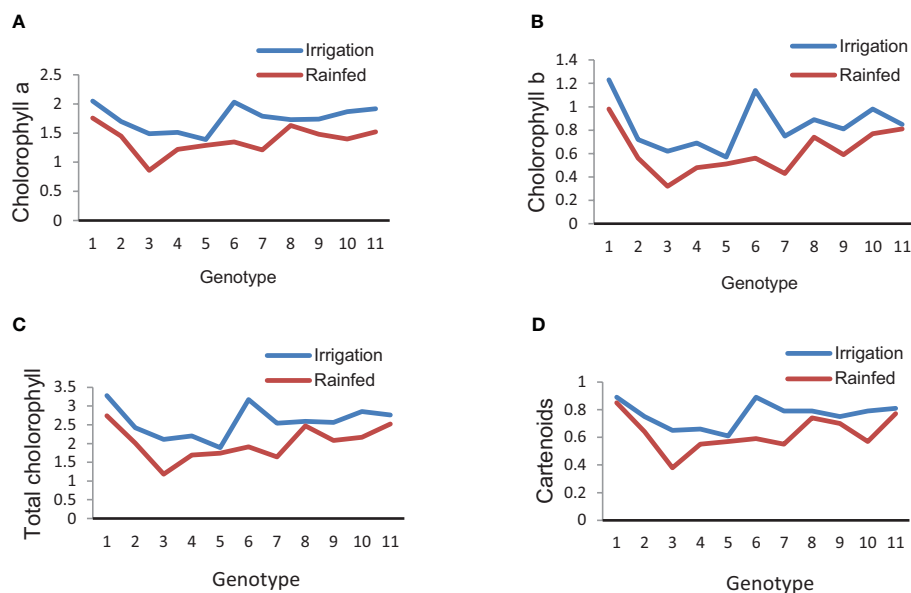


FIGURE 9

Mean comparison for interactions of genotypes x chlorophyll. (A) Average of chlorophyll a under irrigation and rainfed conditions. (B) Average of chlorophyll b under irrigation and rainfed conditions. (C) Average of total chlorophyll under irrigation and rainfed conditions. (D) Average of carotenoids under irrigation and rainfed conditions.

organs, balancing the distribution of photosynthetic materials, and more efficiently using the remaining leaf surfaces (Lopes and Reynolds, 2010; Emam et al., 2013).

The observed diversity in remobilization values from the stems indicates that some genotypes (due to the stronger sink) send more carbohydrates to the grains by stimulating the mechanism of remobilization (Table 4). In this case, a drought-tolerant genotype had a stronger capacity for accumulation and higher remobilization efficiency of pre-anthesis stem water-soluble carbohydrate reserves under terminal drought, resulting in better grain filling and effective compensation for the loss of grain weight, especially in lower internodes (Liu et al., 2020). Saeidi et al. (2012) suggested that the amount of remobilization of storage materials through the stem nodes is higher in resistant cultivars, especially in drought stress conditions. The noteworthy point in this study was the significant increase in SW in all investigated lines in RHS treatment by 22% and 18% in irrigation and rainfed conditions, respectively. With the reduction of the physiological sinks, the current photosynthesis has provided the grain requirement, and the surplus photosynthetic materials have been stored in the stem (Table 6). For instance, genotype 9, which has the highest increase in remobilization from the stem under RAL in irrigation (0.31 g/stem) and rainfed (0.27 g/stem), was ranked second in terms of grain weight and GNS in both conditions (Table 8). This shows the compatibility and resistance of these genotypes to adverse environmental conditions. Of course, this compatibility was not observed in the high-yielding Pishgam cultivar. The amount of remobilization during environmental stress determines the final grain yield (Najafian and Shabani, 2010).

Also, by removing the leaves and reducing the source size, part of the deficit of photosynthetic materials may be compensated by increasing the remobilization from the vegetative organs such as

spike straw. In our study, the remobilization values from spike straw to grains in CH (without S-S manipulation) were 0.38 and 0.43 g/spike in irrigation and rainfed conditions, respectively. Therefore, remobilization from the spike straw compared to the stem on a smaller scale can effectively fill the grains. Overall, when the photosynthetic capacity is reduced due to drought stress or source reduction (leaves), grain filling is significantly dependent on the remobilization from storage organs such as stems and spikes straw, and if the size of the sink is reduced, the survival of storage compounds increases. Removing a part of the spike (reduction of sink size) reduces the need for photosynthetic materials and remobilization from the spike straw to the grains. According to Table 9, line 3 showed the highest increase in remobilization from spike straw in RFL (0.13 g/spike) and RAL (0.32 g/spike) in rainfed conditions. It had the least effect of drought stress on GWS (8.90%) compared to irrigation conditions. This shows the important role of intensification of remobilization from the spike straw in grain filling under critical conditions (Table 4). At the same time, genotypes 1, 4, and 6, which had the lowest increase in remobilization from spike straw in both RFL and RAL treatments in rainfed conditions, were more severely affected by drought stress (Table 9). Generally, source reduction through leaf removal treatments leads to a more favorable utilization of the storage materials in spike straw. The amount of remobilization from spike straw to grains in RAL compared to RFL showed a double increase in irrigation and rainfed conditions. The remobilization from vegetative organs to grains moderates the effect of severe defoliation on grain growth (Emam and Niknejad, 2004).

It is noteworthy that the genotypes in irrigation conditions could increase the remobilization by 0.27 g per plant in RAL, but in RFL, only 0.11 g of these reserve materials was used for remobilization; therefore, it can be said that due to the energy required for decomposition and

remobilization from the stem and spike straw to the grain, most of these compounds are transferred to the grain only in critical conditions.

4.3 Changes in photosynthesis under RHS treatment

Due to competition for assimilates among reproductive organs, it is an important approach to control the source–sink ratio to reduce this competition by removing the reproductive organs and consequently the plant's reproductive potential and yield (Wu et al., 2022). In the present study, the reduction of total photosynthesis due to the halving of grain demand (under RHS treatment) was only 0.37 g instead of 0.97 g (half spike weight) per spike, indicating a negative feedback due to the saturation of photosynthetic materials in the remaining half of the spike (Table 6). This shows that photosynthesis decreased by 38%. Also, in rainfed conditions, the production of photosynthetic materials decreased by only 0.23 g per plant, equivalent to 29% of the weight of half spike (demand reduction). This indicates the presence of stronger sinks in vegetative organs compared to grains for the uptake of photosynthetic materials when the source–sink ratio increases. The source–sink relationship analysis of wheat after anthesis showed that the sink capacity affects the production and distribution of photosynthetic products, and a larger sink capacity can promote the leaf photosynthetic potential and transport of photosynthetic products to spike (Kumar et al., 2017). The amount of dry matter accumulation after anthesis may affect the grain weight, indicating that there was a feedback regulation between the sink and the source after wheat anthesis, and the source can affect the enrichment of the sink (Asseng et al., 2017; Ibrahim et al., 2021). Moreover, the distribution of assimilates was affected by the source–sink ratio. The proportion of assimilates allocated to the spike (sink) was relatively small when the source–sink ratio was large (Abeledo et al., 2020). Moreover, it shows the need to pay attention to increasing the size of the sink to improve grain yield, especially in irrigation conditions if the source is increased. Several researchers proposed analyzing the wheat yield in terms of sink capacity and the degree of sink limitation (Abbate et al., 2005; Lázaro et al., 2010; Alonso et al., 2018), finding that the source for grain filling becomes a limiting factor when the sink capacity increases. Grain yield was highly associated with sink capacity, grain number, biomass, SPAD values, and leaf area index during grain filling, indicating a higher degree of source limitation with an increase in sink capacity. Therefore, source limitation should be taken into account by breeders when sink capacity is increased, especially under non-limiting conditions (Wu et al., 2022). There was a positive relationship between the stem remobilization with the GWS ($r = 0.699^*$) and the GNS ($r = 0.657^*$) and also between the spike straw remobilization with the GWS ($r = 0.605^*$) and the GNS ($r = 0.607^*$) in rainfed conditions. This shows the role and importance of carbohydrate transfer in traits affecting yield (Table 5).

4.4 Remobilization of water-soluble carbohydrates in the stem

During the early grain filling, if the current leaf photosynthesis is unable to thoroughly meet the sink/grain demand, part of the required photo-assimilates for the grain filling would be supplied by the dry matter remobilization from the lower internodes, which have already reached their maximum weight. Also, under terminal drought stress, stem carbohydrate reserves become the major source of grain filling as leaf photosynthesis ceases (Zhang et al., 2015). Research has shown that reserve pools can potentially contribute to approximately 20% of the final grain weight and up to 50% of the grain yield under favorable conditions and drought stress during the grain-filling period, respectively (Hou et al., 2018). Also, a positive and significant correlation has been reported between grain weight per main spike and remobilization rate in wheat under terminal drought stress (Li et al., 2020).

In this study, the average remobilization efficiency (%) in irrigation and rainfed conditions was 53.10% and 59.18%, respectively, which increased by 11.45% in rainfed conditions (Table 10). Also, the contribution of WSC remobilization in grain yield (%) in rainfed conditions has increased by 19% compared to irrigation conditions. These results show the effect and importance of WSC remobilization in drought stress conditions (Table 10). This contribution of the sucrose flux from the stem to the grain seems to be more important for yield maximization under drought conditions (Joudi et al., 2012). Nevertheless, other findings demonstrated a significant increase in the rate and efficiency of assimilate remobilization from the stem internodes under drought stress (Ma et al., 2014; Vosoghi Rad et al., 2022). In addition, the amount of remobilization among the wheat population can be influenced by the amount of accumulated reserves in the stem as well as the remobilization efficiency, which, in turn, depends on the strength of the sink (Thapa et al., 2022). Hence, it can be argued that the remobilization amount and efficiency are differently influenced by the cultivar and the severity of the drought stress.

4.5 Effect of drought stress on the chlorophyll content of flag leaf

Furthermore, drought stress decreased the contents of chlorophyll in all genotypes (Table 11; Figures 9A–D). Researchers registered slight flag leaf senescence after anthesis in the optimal conditions, which can be accelerated by drought conditions (Liu et al., 2009), while others affirm that post-anthesis drought significantly accelerated chlorophyll loss (Martinez et al., 2003). Drought stress can destroy or reduce chlorophyll content and inhibit its synthesis (Hassanzadeh et al., 2009). Decreased yield in drought-sensitive genotypes might be due to a reduction in chlorophyll as well as photosynthetic parameters (Perdomo et al., 2017). Drought-tolerant genotypes retained many photosynthetic pigments under drought stress (Epee Misse, 2018). Also, it has been

observed that the chlorophyll content was highly maintained during the initial grain-filling period, and their photosynthetic capacity gradually decreased after this period (Fan et al., 2021). The significant relationship between chlorophyll content with resource limitation seems to be due to stronger sinks in these genotypes.

5 Conclusion

Some levels of source limitation were observed in all genotypes, and vegetative organs seem to have a larger sink than grains to uptake photosynthetic materials when the source-sink ratio increases. However, high-yielding genotypes had more severe source limitations, and low-yielding genotypes had more relative sink limitations. Therefore, source limitation does not necessarily occur due to the smallness of the source, and in high-yielding genotypes, it may be due to the largeness of the sinks. Hence, to increase the yield potential of high-yielding cultivars, the size of photosynthetic sources and, in cultivars with lower yields, the size of sinks should be improved. Also, by RFL, increasing remobilization from vegetative organs (stem and spike straw) to grains moderates the effects of source limitation, and it compensates decrease in grain weight. This role of remobilization was more intense in RAL. Furthermore, depending on the cultivars and environmental conditions, the amount of WSC retransplantation was calculated from 22 to 81 g/m². Accordingly, WSC remobilization in critical conditions shows stronger positive effects on grain yield. This is also recommended to be considered in physiological and molecular studies focusing on carbohydrate remobilization of wheat stems. Also, by RHS, the decrease in the production of photosynthetic materials was only equivalent to 38% and 29% of the expected values in both conditions, which shows the presence of strong sinks in vegetative organs (stem and spike straw) compared to grains, and it is necessary to pay attention to them in order to improve wheat genotypes. Taken together, investigating the S-S relationship along with the ability to remobilization in optimal and critical conditions displayed a promising perspective in decreasing growth limitations and selecting potential genotypes in wheat in temperate regions.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

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Author contributions

KE: Data curation, Formal analysis, Writing – original draft. AS: Conceptualization, Funding acquisition, Resources, Supervision, Writing – original draft. FH: Investigation, Validation, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. The authors are indebted to the University of Kurdistan (UOK) for the financial support given to carry out this study.

Acknowledgments

The authors sincerely thank Mosayeb Norozi and Rasol Jaliliyan of the Islamabad-e-Gharb Agricultural Research Station for their help in implementing the experiments. They also deeply thank Reza Amiri and Layegh Moradi for their help and support.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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OPEN ACCESS

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RECEIVED 23 April 2024

ACCEPTED 31 May 2024

PUBLISHED 18 June 2024

CITATION

Amiriyani Chelan Z, Amini R and
Dabbagh Mohammadi Nasab A (2024)
Optimizing fenugreek (*Trigonella foenum-
graecum* L.) oil yield and compositions in
intercropping through growth-promoting
bacteria and mycorrhiza.
Front. Agron. 6:1422236.
doi: 10.3389/fagro.2024.1422236

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Optimizing fenugreek (*Trigonella foenum-graecum* L.) oil yield and compositions in intercropping through growth-promoting bacteria and mycorrhiza

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Introduction: Biofertilizers and intercropping are two main components in sustainable production systems.

Materials and methods: A two-year (2020–2021) study was conducted in East Azarbaijan, Iran, to evaluate the effects of arbuscular mycorrhizal fungi (AMF), growth-promoting bacteria (GPB) and chemical fertilizer (CF) on fenugreek (*Trigonella foenum-graecum* L.) (F) oil yield and compositions in intercropping with Moldavian balm (*Dracocephalum moldavica* L.) (MB). The cropping patterns included MB sole cropping, fenugreek sole cropping (F) and replacement intercropping ratios consisted of Moldavian balm : fenugreek (MB:F (1:1)), MB:F (2:2) and MB:F (4:2) and additive intercropping of MB:F (100:50).

Results: For both years, among the intercropping patterns, the highest seed and oil yields were obtained in MB:F (100:50) intercropping pattern treated with CF and AMF+GPB. In all cropping patterns except MB:F (4:2), the highest anthocyanin, total flavonoid, and mucilage contents were observed in plants received AMF+GPB. At all treatments, the linoleic, oleic, and linolenic acid were the main components of fenugreek oil. In MB:F (1:1), (2:2), (4:2), and (100:50) intercropping patterns, the linoleic acid content in AMF+GPB treatment, increased by 9.45%, 6.63%, 15.20%, and 7.82%, respectively, compared with sole fenugreek. The highest total land equivalent ratio (LERT) values were obtained in 2021 and MB:F (100:50) intercropping pattern treated with CF (1.70) and AMF+GPB (1.63).

Conclusions: In general, it could be concluded that MB:F (100:50) intercropping pattern treated with AMF+GPB improved the oil yield and unsaturated fatty acid contents of fenugreek compared with sole cropping and could be recommended in sustainable production systems.

KEYWORDS

fenugreek, fatty acid composition, legume-based intercropping, total flavonoid, mucilage

1 Introduction

Fenugreek (*Trigonella foenum-graecum* L.) is one of the oldest medicinal plants in the world, which belongs to the Fabaceae family (Zandi et al., 2017). This medicinal plant is native to an area extending from Iran to northern India but is now cultivated in China, Greece, Ukraine, and north and east Africa (Petropoulos, 2002). Also, in central regions of Iran, different species of this plant are used for traditional Persian medicine (Jhajhria and Kumar, 2016). This plant is recommended for arid and semi-arid regions of Asia, sub-Saharan Africa, and Latin America as a low input and annual dryland legume. In Iran, fenugreek could be used for commercial production for small-scale farms with low capacity for investment (Basu et al., 2017). It has been reported that fenugreek is useful for humans in the treatment of a number of diseases, including reducing blood glucose, blood cholesterol, hair loss, liver ailments, and skin eruptions (Camlica and Yaldiz, 2024), because it contains trigonelline, diosgenin, flavonoid, and other compounds (Zandi et al., 2017). Moldavian balm (MB) (*Dracocephalum moldavica* L.) is an herbaceous and annual medicinal plant, native to Central Asia and domesticated in Central and Eastern Europe (Vafadar-Yengeje et al., 2019; Amini et al., 2020). All organs of this plant contain essential oil, and their content varies depending on organ type, nutrients availability, and ecological factors (Hussein et al., 2006).

One of the main goals of agricultural systems is to achieve production stability and increase the productivity of agricultural ecosystem, through intercropping different compatible crops (Banik and Sharma, 2009). Intercropping system is aimed at creating an ecological balance, using more resources; reducing the damage of pests, diseases, and weeds; and reducing soil erosion and economic risk of production by increasing the quantity and quality of performance against time and place (Marastoni et al., 2019). The differences in nutrient uptake by different plants is important when designing intercropping systems and the use of legumes in intercropping is an effective way to compensate for nitrogen deficiency in the soil and increase production (Raza et al., 2021; El-Mehy et al., 2023). Hence, the implementation of the intercropping system of medicinal plants, one of its components is nitrogen fixation, can play a more effective role in using environmental resources and increase the productivity of the cropping system (Yaseen et al., 2014; Sakhavi et al., 2017a, Sakhavi et al., 2017b). Few studies have shown that intercropping system can affect the production, qualitative aspects, and chemical compositions of medicinal plants (Weisany et al., 2015; Vafadar-Yengeje et al., 2019; Amini et al., 2020; Rezaei-Chiyaneh et al., 2021).

In sustainable agricultural systems, one of the solutions to improve and maintain soil fertility is the use of internal (in-farm) inputs, including beneficial soil microorganisms that are known as biofertilizers (Sharma et al., 2013; Amini et al., 2017). These microorganisms are of special importance in sustainable agriculture with the aim of stimulating the nutrients cycle and reducing the need for chemical fertilizers (CFs) (Turan et al., 2010; Sarikhani and Amini, 2020). Among the biological fertilizers are arbuscular mycorrhizal fungi (AMF), which are able to increase the effective surface of the roots by creating a wide network and provide access to a large volume of soil (de Assis et al., 2020). Earlier studies have shown that mycorrhizal fungi cause significant changes in the quantity and quality

of secondary metabolites of medicinal plants (Merlin et al., 2020). Using the mycorrhizal fungi in intercropping of dill (*Anethum graveolens* L.) with common bean (*Phaseolus vulgaris* L.) increased the essential oil yield of dill (Weisany et al., 2015). Also, the positive effect of mycorrhizal fungus on essential oil yield of dill and carum (*Trachyspermum ammi* Sprague) (Kapoor et al., 2002) and chamomile (*Matricaria chamomilla* L.) (de Almeida et al., 2020) have been reported. Atmospheric N₂ can be fixed in the form of nitrate and ammonium ion by certain strains of *Azospirillum*, *Azotobacter*, and *Rhizobium*, which can be taken up by the plants, thereby improving growth (Sahoo et al., 2012). *Azotobacter* serves as a biofertilizer for important crops, such as wheat, barley, sesame, rice, maize, and sunflower. In addition to N₂ fixation, *Azotobacter* is as a rich source of phytohormones such as gibberellins (GA) and indole acetic acid (IAA) (Dar et al., 2021). *Azospirillum* can enhance plant growth, development, and yield by increasing N₂ status of the plant that could be attributed to different mechanisms, such as auxin synthesis and biological N₂ fixation (Sahoo et al., 2012). Therefore, in production of medicinal plants, using biofertilizers could improve the quantity and quality of oil constituents, which is compatible with the goals of sustainable agricultural. Due to the necessity of evaluating the ecological dimensions of intercropping in sustainable production, this experiment was conducted with the aim of evaluating the oil yield and compositions of fenugreek in sole and intercropping with MB under biofertilizer [growth-promoting bacteria (GPB) and mycorrhiza] treatments.

2 Materials and methods

2.1 Experimental site, design, and field practice

This research was conducted in Maragheh City in East Azarbaijan province, Iran (latitude 37°4' N, longitude 46°26' E, altitude 1478 m above sea level) in 2020 and 2021 growth seasons. The climatic data of monthly total precipitation and mean temperature of the experimental site during the growth seasons of 2020 and 2021 are presented in Table 1. The soil characteristics of the experimental field at a depth of 0–30 cm are presented in Table 2.

The 5 × 3 factorial experiments were carried out based on randomized complete block design with three replications in 2020 and 2021. The cropping pattern (first factor) consisted of five levels: MB sole cropping, fenugreek sole cropping (F), and replacement intercropping ratios including 1 row of MB + 1 row of fenugreek (MB:F (1:1)), 2 rows of MB + 2 rows of fenugreek (MB:F (2:2)), and 4 rows of MB + 2 rows of fenugreek (MB:F (4:2)) and additive

TABLE 1 Monthly total precipitation and mean temperature in 2020 and 2021 growing seasons in the experimental area.

	Year	May	June	July	August	September
Total precipitation (mm)	2020	14.23	2.03	3.3	1.27	0
	2021	12.71	0	1.2	0	0
Mean temperature (°C)	2020	20.2	25.5	29	26	24.8
	2021	22.5	28.7	29.5	29.1	24.3

TABLE 2 Physicochemical properties of the soil of experimental area in depth of 0–30 cm.

Parameter	Soil texture			pH	Organic matter (%)	EC (dS m ⁻¹)	Total N (%)	P (mg kg ⁻¹)	K (mg kg ⁻¹)
	Sandy loam								
Value	Clay (%)	Silt (%)	Sand (%)	7.28	1.0	1.76	0.033	9.3	620
	9	27	64						

intercropping of MB + fenugreek MB:F (100: 50) (100% density of MB + 50% density of fenugreek planted between MB rows). The fenugreek is dominated crop, and the MB is dominating crop. The fertilizer treatment (second factor) consisted of three levels: 100% CF, application of AMF, and combined application of AMF and GPB (AMF+GPB). CF treatment was 50 kg ha⁻¹ urea and 80 kg ha⁻¹ triple superphosphate (according to soil test results), which were applied at planting time. Myco-Root bio-fertilizer contains arbuscular mycorrhiza fungi (AMF) of *Glomus mosseae*, *Glomus intraradices*, and *Glomus etunicatum* with count 10⁷ to 10⁸ CFU/g is provided by Zist Fanavar Pishtaz Varian Company, Karaj, Iran. This bio-fertilizer is an easy-to-use powder form that is used for crops as seed inoculation. According to the manufacturer's instructions, 1 kg of MB and fenugreek seeds were placed in the shade on a clean surface, and after spraying a small amount of water on them, 40 g of AMF bio-fertilizer was added and mixed thoroughly, so that all the seeds were covered with a layer of bio-fertilizer. For inoculating the GPB, Biofarm bio-fertilizer used contained *Azospirillum brasilense* and *Azotobacter chroococcum* bacteria with a population of 2 × 10⁷ CFU/g and was provided by Nature Biotechnology Company (Biorun) Karaj, Iran. According to the manufacturer's instructions, 1 kg of MB and fenugreek seeds were inoculated with 40 mL of Biofarm and then planted. Also, the seeds of fenugreek were inoculated with *Sinorhizobium meliloti* for nitrogen fixing through symbiosis.

The deep mouldboard ploughing (25–30 cm) was used in the spring for seedbed preparation, which was followed by disk harrowing. The seeds of fenugreek and MB were planted manually at densities of 500,000 and 320,000 plants ha⁻¹, respectively. In sole cropping and intercropping patterns, both crops were planted with 25 cm row space on 4 May 2020 and 15 May 2021. The size of the experimental plots in sole fenugreek, sole MB, replacement intercropping patterns of MB:F (1:1), MB:F (2:2)) and additive intercropping of MB:F (100: 50) were 3 m (12 rows) wide × 3 m long. In replacement intercropping of MB:F (4:2), the size of the experimental plot was 4 m (16 rows) wide × 3 m long. The furrow irrigation was done after planting of both crops with 5-day intervals till seed maturity. During the growing season, the weeds in experimental plots were removed 3, 5, 7, 9, and 11 weeks after sowing by hand weeding. There was no need for pesticide application in the experimental field.

2.2 Fenugreek growth, seed, and oil yield

In each plot, 10 plants were randomly selected after removing the marginal effects (side rows and half a meter from the sides of the

middle rows), and the selected plants were tagged before flowering stage (40 days after sowing). In both years, at maturity stage on 8 August 2020 (96 days after sowing) and 22 August 2021 (99 days after sowing), the fenugreek height was measured with a steel rule with the least count of 0.5 mm. To measure leaf chlorophyll content index (SPAD), chlorophyll content Meter SPAD-502 (Konica Minolta) device was used in vegetative growth stage (30 and 32 days after sowing in 2020 and 2021, respectively) and flowering stage (49 and 51 days after sowing in 2020 and 2021, respectively). Five plants were randomly selected from each plot, and the chlorophyll content index was recorded in three new full expanded leaves from upper, middle, and lower part of each plant and the average of the recorded values for two stages were used in data analysis (Vafadar-Yengeje et al., 2019). To determine fenugreek seed yield, in maturity stage, the plants in the middle rows of 1 m⁻² area of each plot were harvested and dried at room temperature for 48h, and after threshing, the seed yield was determined. In order to extract the oil, 10 g of crushed seeds of each treatment were packed in Whatman paper, and then oil extraction was done using Soxhlet apparatus and 400 mL n-hexane solvent for 2.5h at 70°C. After 8h, the solvent was evaporated from the oil using a rotary and by measuring the amount of oil; it was measured as a percentage (oil content) (Fotahi Chiyaneh et al., 2022). Finally, the oil samples were stored at 4°C until the identification of chemical compounds with a gas chromatography–mass spectrometry (GC–MS) device. Oil yield was calculated using Equation 1:

$$\text{Oil yield (kg ha}^{-1}\text{)} = \text{oil content (\%)} \times \text{seed yield (kg ha}^{-1}\text{)} \quad (1)$$

2.3 Gas chromatography–mass spectrometry

The seed oil of fenugreek was analyzed using a GC–MS (Agilent 6890N, USA) with HP-5 MS column (30-mm diameter of tubular column, 0.25-mm internal diameter, and 0.25-μm thickness of film) as described with Fotahi Chiyaneh et al. (2022). The fatty acid methyl esters were prepared using the method described by Heidari et al. (2020). Two hundred microliter of the 2.0 M solution of methanolic potassium hydroxide was added to 50 mg of the sample in 2 mL n-hexane. The mixture was vigorously vortexed for 1 min and allowed to stand in a dark place until it becomes separate into two phases. After the upper hexane layer became transparent, 1 μL was injected into the GC–MS column. The identification of the chemical compounds of the oil was done by matching the mass spectra obtained of the sample through comparison with the mass spectrum report provided by Wiley

7.0 and Adams (Adams, 2001). The GC–MS analysis was done for fenugreek seed oil obtained in all treatments.

2.4 Anthocyanin, total flavonoid, and mucilage contents

In order to measure the amount of anthocyanin in fenugreek oil, 0.02 g of dry seeds obtained in all experimental plots (treatments) were ground with 4 mL of 1 M hydrochloric acid solution containing methanol in a porcelain mortar and, after 24 h of storage in the refrigerator, the obtained solution was centrifuged for 10 min at 13,000 rpm. Then the upper phase was removed and the absorbance of the solutions was measured at 530 and 657 nm with a spectrophotometer (Mita et al., 1997). One molar hydrochloric acid of methanol solution was also used as a control and the amount of anthocyanin was obtained using Equation 2:

$$A = A_{530} - (0.25 \times A_{637}) \quad (2)$$

where A is the absorption of the solution and subscript numbers indicate the wavelengths in which the absorption was measured.

To measure the total flavonoid content of fenugreek, 0.1 g of dry seeds obtained in all experimental plots (treatments) were ground with 5 mL of ethanol in a porcelain mortar and then centrifuged at 10,000 rpm for 5 min. Then, 500 μ L was removed from the upper phase and 1.5 mL of ethanol, 100 μ L of 10% aluminum chloride, 100 μ L of 1 M potassium acetate, and 2.8 mL of distilled water were added, and then was kept for 40 min at room temperature. Then the absorbance of the solutions was measured at 415 nm compared to the control without herbal extract (Chang et al., 2002). Finally, by placing the absorption value of the samples in the standard curve equation of quercetin, the amount of total flavonoid was measured in terms of mg of quercetin per g of seeds dry weight. The mucilage content was measured by the method of Kalyanasundaram et al. (1982). In all experimental plots (treatments), the combination of 1 g of dry seed and 10 mL of 0.1 normal hydrochloric acid solution was heated until the color of the seed shell changed, and after adding 60 mL of 96% ethyl alcohol, it was kept in the refrigerator for 5 h. After filtering, the sediment was placed in a 50°C oven for 12 h. Finally, after weighing, the mucilage content in fenugreek seeds was measured as a percentage.

2.5 Land equivalent ratio

In fenugreek–MB intercropping patterns the land equivalent ratio (LER) values were evaluated using Equations 3 and 4:

$$LER_F = Y_{FI} / Y_F \text{ and } LER_{MB} = Y_{MBI} / Y_{MB} \quad (3)$$

$$LER_T = LER_F + LER_{MB} \quad (4)$$

Where Y_F and Y_{FI} are the fenugreek seed yields in sole cropping and intercropping patterns, respectively, and Y_{MB} and Y_{MBI} are the MB dry herbage yields in sole cropping and intercropping patterns,

respectively. Also, LER_F and LER_{MB} represent the partial LER of fenugreek and MB, respectively, and LER_T is the total LER.

2.6 Statistical analysis

For analysis of variance (ANOVA), the SAS version 9.0.3 package was used. For two growing seasons of 2020 and 2021 and all traits, the combined ANOVA was done based on complete randomized block design with 15 treatments and three replicates. The data of LER_F , LER_{MB} , and LER_T were not subjected to analysis of variance. The experimental data met the assumptions of normality and variance homogeneity, and no transformation was needed. For comparison of the means, the Duncan's multiple range test was used at $p \leq 0.05$.

3 Results

3.1 Fenugreek plant height

The effects of year, cropping pattern, year $\times$ cropping pattern, and fertilizer treatment were significant ($p \leq 0.05$) on fenugreek plant height (Table 3). The interaction effect of year $\times$ cropping pattern (Figure 1) indicated that, in sole fenugreek, MB:F (4:2) and MB:F (100:50) intercropping patterns, the plants in 2020 were taller than those in 2021, while in MB:F (1:1) intercropping, the plants were taller in 2021. In MB:F (2:2) intercropping, the plants heights in 2020 and 2021 were not significantly different. Also the plants that received CF were taller than those in AMF and AMF+GPB treatments (Table 4).

3.2 Leaf chlorophyll content index (SPAD)

The effects of year, cropping pattern, and fertilizer treatment were significant ($p \leq 0.01$) on fenugreek SPAD (Table 3). The SPAD value in 2020 was higher than 2021 (Table 4). The highest (67.24) and lowest (62.78) SPAD values were observed in MB:F (2:2) and MB:F (100:50) intercropping patterns, respectively (Table 4). The SPAD values in plants treated with AMF+GPB and CF treatments increased significantly compared with that in AMF treatment.

3.3 Seed yield (kg/ha)

Fenugreek seed yield was influenced by year, cropping pattern, year $\times$ cropping pattern, fertilizer treatment, and cropping pattern $\times$ fertilizer treatments ($p \leq 0.01$) (Table 3). The interaction effect of year $\times$ cropping pattern (Figure 2) showed that, in all cropping patterns except the MB:F (4:2), the seed yields in 2020 were higher than those in 2021. In MB:F (4:2) intercropping, the seed yields in 2020 and 2021 were not significantly different. The interaction of cropping pattern $\times$ fertilizer (Figure 3) showed that, in sole

TABLE 3 Analysis of variance for effect of cropping system on selected traits of fenugreek under different fertilizer treatments.

Source of variation	df	Plant height	Chlorophyll content (SPAD)	Seed yield	Oil content	Oil yield	Anthocyanin	Flavonoid	Mucilage content
Year (Y)	1	*	**	**	**	**	**	**	**
Block × Y	4	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
Cropping pattern (C)	4	**	**	**	**	**	**	**	**
Y × C	4	**	n.s	**	n.s	**	n.s	n.s	n.s
Fertilizer (F)	2	*	**	**	**	**	**	**	**
Y × F	2	n.s	n.s	n.s	*	n.s	n.s	n.s	n.s
C × F	8	n.s	n.s	**	**	**	n.s	**	**
Y × C × F	8	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
CV (%)		8.6	3.87	6.69	5.9	10.46	8.93	9.5	7.75

n.s, * and **: non -significant and significant at $p \leq 0.05$ and $p \leq 0.01$, respectively.

fenugreek, MB:F (4:2) and MB:F (100:50) intercropping patterns, the seed yields of plants received CF and AMF+GPB treatments were not significantly different, while in MB:F (1:1) and MB:F (2:2) intercropping patterns, the highest seed yields were observed in plants treated with AMF+GPB. Among the intercropping patterns, the highest seed yield was produced in MB:F (100:50).

3.4 Seed oil content (%)

The effects of year, cropping pattern, fertilizer treatment, and interaction effects of year × fertilizer treatment and cropping pattern × fertilizer treatment were significant ($p \leq 0.01$) on fenugreek seed oil content (Table 3). The interaction effect of year × fertilizer treatment (Figure 4) indicated that, in plants treated with CF and AMF+GPB, the oil contents in 2020 were lower than those in 2021. The oil contents of plants treated with AMF were not significantly different in 2020 and 2021. The interaction effect of cropping pattern × fertilizer (Figure 5) indicated that, in sole

fenugreek, the lowest oil content was observed in plants treated with CF, while in MB:F (1:1), MB:F (4:2) and MB:F (100:50) intercropping patterns, the oil contents of plants received CF and AMF+GPB treatments were not significantly different. In MB:F (4:2) intercropping pattern, the plants treated with AMF had higher oil content than those in CF and AMF+GPB treatments (Figure 5).

3.5 Oil yield (kg/ha)

The effects of year, cropping pattern, year × cropping pattern, fertilizer treatments, and cropping pattern × fertilizer treatment were significant ($p \leq 0.01$) on oil yield (Table 3). The interaction effect of year × cropping pattern (Figure 6) indicated that, in all cropping patterns except the MB:F (4:2), the oil yields in 2020 were higher than those in 2021. The mean comparison of interaction effect of cropping pattern × fertilizer treatment (Figure 7) showed that, in sole fenugreek, the highest oil yield was observed in plants treated with AMF+GPB, while in all intercropping patterns, the oil

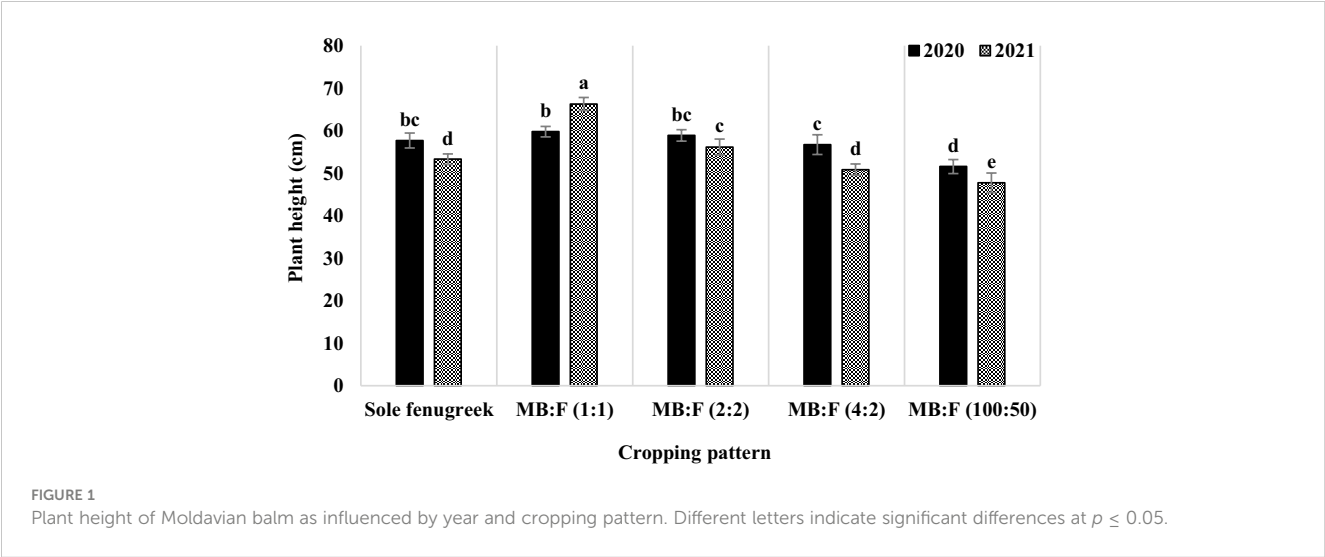


TABLE 4 The effect of year, cropping pattern, and fertilizer treatment on fenugreek height, chlorophyll content, seed yield, oil content, oil yield, anthocyanin, total flavonoid, and mucilage.

Effect	Levels	Plant height (cm)	Chlorophyll content (SPAD)	Seed yield (kg/ha)	Oil content (%)	Oil yield (kg/ha)	Anthocyanin (mg/g)	Total flavonoid (mg EQ/g)	Mucilage content (%)
Year	2020	56.92 ± 0.85 a	67.53 ± 0.49 a	1009.8 ± 62.28 a	3.588 ± 0.063 b	36.244 ± 2.24 a	0.1649 ± 0.0036 b	26.67 ± 0.854 b	13.66 ± 0.27 b
	2021	54.86 ± 1.20 b	63.59 ± 0.46 b	758.3 ± 46.36 b	3.907 ± 0.036 a	28.694 ± 1.84 b	0.1891 ± 0.0042 a	29.70 ± 0.855 a	15.14 ± 0.43 a
Cropping pattern	Sole fenugreek	55.49 ± 1.17 c	66.79 ± 0.89 ab	1308 ± 51.58 a	3.621 ± 0.083 b	40.91 ± 1.67 a	0.1546 ± 0.0056 b	28.27 ± 1.079 b	14.52 ± 0.62 bc
	MB:F (1:1)	63.03 ± 1.24 a	65.13 ± 0.88 b	838.9 ± 35.32 c	3.784 ± 0.082 a	30.78 ± 1.18 d	0.1896 ± 0.006 a	28.99 ± 1.324 b	15.42 ± 0.51 a
	MB:F (2:2)	57.53 ± 1.67 b	67.24 ± 0.73 a	879.9 ± 51.87 c	3.921 ± 0.103 a	33.38 ± 1.92 c	0.1867 ± 0.0072 a	25.03 ± 0.825 c	14.23 ± 0.46 c
	MB:F (4:2)	53.78 ± 1.48 c	65.76 ± 0.82 ab	295.3 ± 12.91 d	3.523 ± 0.092 b	10.04 ± 0.33 e	0.1634 ± 0.0040 b	24.00 ± 1.327 c	12.77 ± 0.32 d
	MB:F (100:50)	49.65 ± 1.45 d	62.78 ± 0.77 c	1098 ± 55.82 b	3.886 ± 0.049 a	41.22 ± 2.04 b	0.1908 ± 0.0057 a	34.49 ± 0.876 a	15.06 ± 0.79 ab
Fertilizer treatment	CF	57.95 ± 1.62 a	66.17 ± 0.68 a	922.8 ± 73.48 b	3.622 ± 0.068 b	33.00 ± 2.57 b	0.1609 ± 0.0041 c	23.95 ± 0.875 c	12.55 ± 0.24 c
	AMF	55.48 ± 1.40 b	64.15 ± 0.67 b	755.5 ± 62.91 c	3.959 ± 0.056 a	29.06 ± 2.37 c	0.1723 ± 0.0046 b	28.72 ± 1.065 b	14.12 ± 0.28 b
	AMF+GPB	54.26 ± 1.21 b	66.36 ± 0.64 a	973.8 ± 71.39 a	3.661 ± 0.068 b	35.35 ± 2.78 a	0.1978 ± 0.0047 a	31.81 ± 0.761 a	16.53 ± 0.48 a

F, fenugreek; MB, Moldavian balm. CF, AMF, and AMF+GPB are 100% chemical fertilizers, arbuscular mycorrhizal fungi, and growth-promoting bacteria, respectively. Different letters indicate significant differences at $p \leq 0.05$.

yields in plants received CF and AMF+GPB were not significantly different. Among the intercropping patterns, the highest oil yields were observed in MB:F (100:50).

3.6 Oil composition (GC–MS)

The effects of intercropping patterns and fertilizer treatments on oil composition were evaluated through GC–MS analysis, and it was observed that the fenugreek seed oil contains eight main fatty acids, which constitute 92.6%–97.29% of total composition of the oil (Table 5). The main components of fenugreek seed oil were linoleic acid (39.21%–21.21%), oleic acid (23.65%–18.79%), linolenic acid (31.21%–21.17%), palmitic acid (12.14%–7.65%) and stearic acid (13.7%–25.25%), respectively. In all fertilizer treatments, the lowest content of linoleic acid was obtained in sole crop, and increased in all intercropping patterns and the highest value was observed in plants treated with MB:F (4:2) (Table 5). In all cropping patterns except MB:F (1:1), the plants received AMF and AMF+GPB fertilizer treatments had higher linoleic acid contents than CF treatment.

In all fertilizer treatments, the contents of oleic acid improved in MB:F (2:2) compared to other cropping patterns, so that the highest value was observed in this cropping pattern when treated with AMF +GPB (23.65%). The lowest content of oleic acid was observed in plants received AMF+GPB and MB:F (100:50) intercropping. In intercropping patterns except MB:F (100:50), the oleic acid contents under AMF and AMF+GPB treatments were higher than those in CF treatment. The highest content of linolenic acid was observed in MB:F (4:2) intercropping treated with AMF+GPB, which increased by 17.39% than sole crop. Also, the lowest content of linolenic acid was related to the plants received AMF+GPB in MB:F (1:1). The linolenic acid contents under AMF+GPB treatment was higher than those in CF and AMF fertilizer treatments except for MB: F (1:1) and (100:50) intercropping patterns (Table 5).

The highest contents of palmitic acid were obtained in sole crop (11.89, 14.12, and 10.99% under CF, AMF and AMF+GPB treatments, respectively). The lowest content of palmitic acid (7.56%) was obtained in plants received AMF and MB:F (4:2) intercropping, which decreased by 59.44% compared with that in sole fenugreek. In sole fenugreek, MB:F (1:1) and (100:50) cropping patterns, plants received AMF had higher palmitic acid contents compared to those in AMF+GPB and CF treatments, while in MB:F (2:2) and (4:2), the plants treated with CF had higher contents of palmitic acid. The highest contents of stearic acid were obtained in sole fenugreek and among the fertilizer treatments the highest value (13.2%) was observed under AMF treatment. In MB:F (1:1), (2:2) and (100:50) intercropping patterns, the content of stearic acid under CF treatment was higher than those in other fertilizer treatments, while in MB:F (4:2), the highest content of stearic acid was obtained in plants treated with AMF.

3.7 Anthocyanin (mg/g)

The content of anthocyanin in fenugreek seeds was significantly affected by year, cultivation pattern, and fertilizer treatments ($p \leq$

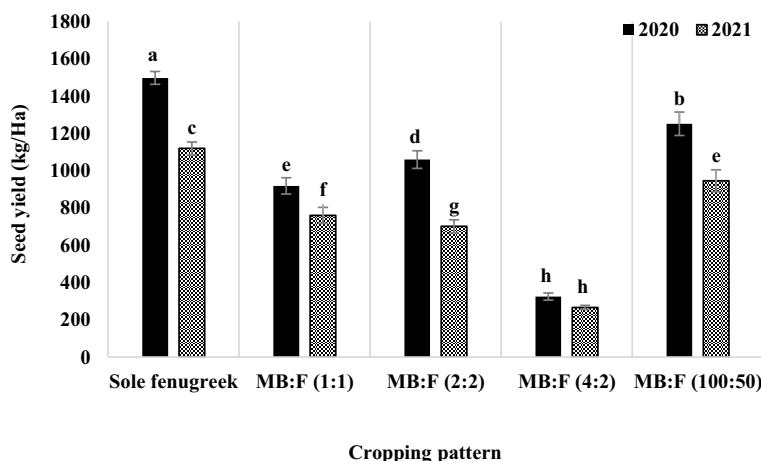


FIGURE 2

Fenugreek seed yield as influenced by year and cropping pattern. Different letters indicate significant differences at $p \leq 0.05$.

0.01) (Table 3). The anthocyanin content in 2021 was higher than 2020. In MB:F (100:50), (1:1), and (2:2) cropping patterns, the anthocyanin contents increased significantly compared with sole fenugreek and MB:F (4:2). Among the fertilizer treatments, the plants treated with AMF+GPB had the highest content of anthocyanin (Table 4).

3.8 Total flavonoid (mg EQ/g)

The effects of year, cropping pattern, fertilizer treatments, and the interaction effect of cropping pattern $\times$ fertilizer treatment were significant ($p \leq 0.01$) on total flavonoid content of fenugreek seeds (Table 3). The content of total flavonoid in 2021 was higher than 2020 (Table 4). The mean comparison for interaction effect of cropping pattern $\times$ fertilizer treatment (Figure 8) showed that, in all cropping patterns, except in MB:F (4:2), the highest contents of total

flavonoid were observed in plants treated with AMF and AMF +GPB. In MB:F (4:2) intercropping, the total flavonoid content in plants that received AMF+GPB was higher than those of CF and AMF.

3.9 Mucilage content (%)

The mucilage content of fenugreek seed was also affected by year, cropping pattern, fertilizer treatment, and interaction effect of cropping pattern $\times$ fertilizer treatments ($p \leq 0.01$) (Table 3). The mucilage content increased in 2021 compared with that in 2020 (Table 4). In all cropping patterns (except in MB:F (4:2)), the mucilage contents increased significantly in plants treated with AMF+GPB, compared with those in CF and AMF (Figure 9). In MB:F (4:2) intercropping, the mucilage contents in plants received AMF and AMF+GPB were not significantly different.

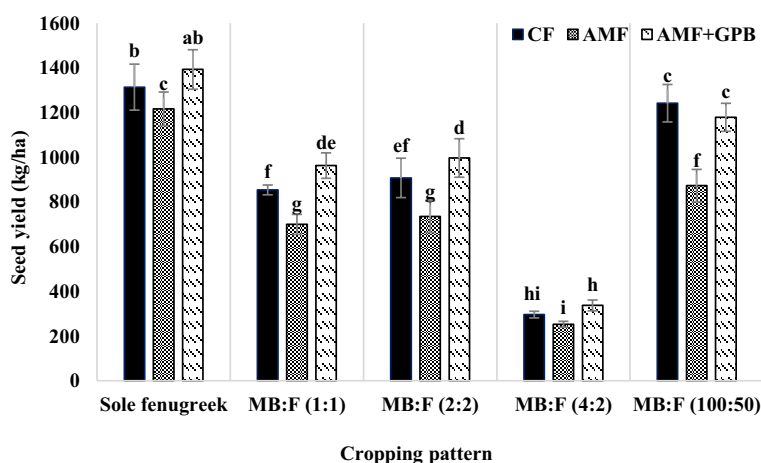


FIGURE 3

Fenugreek seed yield as influenced by cropping pattern and fertilizer treatments. Different letters indicate significant differences at $p \leq 0.05$.

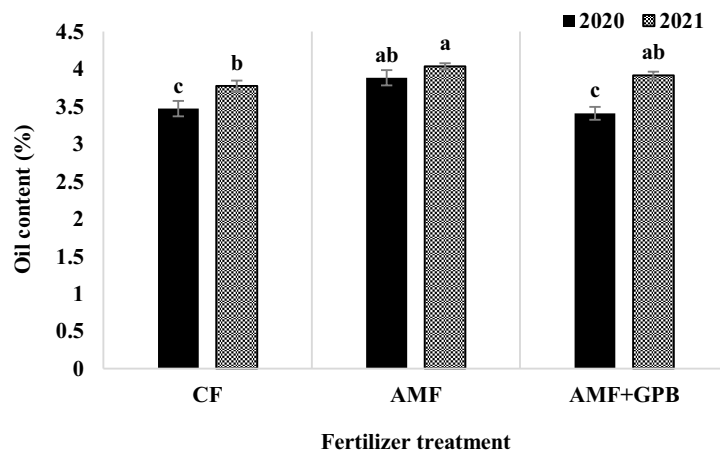


FIGURE 4

Fenugreek oil content as influenced by year and fertilizer treatments. Different letters indicate significant differences at $p \leq 0.05$.

3.10 LER of intercropping patterns

The LER_T index of all intercropping patterns were higher than 1.0 in both experimental years (Table 6). In general, LER_T values in MB:F (100:50) were higher than those in MB:F (1:1), (2:2) and (4:2) intercropping patterns. In both years, the MB:F (100:50) intercropping pattern treated with CF had the highest LER_T (1.58 and 1.70 for 2022 and 2021, respectively) and the AMF+GPB fertilizer treatment was the next. Comparison of LER_F and LER_{MB} (partial LER_s) showed that, in most treatments [except MB:F (4:2)], LER_F values were higher than those of LER_{MB} , which indicates that the intercropping had positive effect on fenugreek.

4 Discussion

4.1 Fenugreek plant height

The plant height is one of the characteristics that is affected by the plant growth conditions, and higher precipitation in 2020

could be the reason for greater fenugreek height in this year compared with 2021. The fenugreek heights in MB:F (1:1) and (2:2) cropping patterns were higher than sole fenugreek, which could be due to increase in competition between plants for light in intercropping compared to sole cropping. Shading of MB likely increased the auxin concentration in fenugreek plants and increased the plant height (Agegnehu et al., 2006). The reason for decrease in plant height in MB:F (100:50) intercropping could be attributed to competition between plants for limited resources (water, nutrients, and light), which has caused a decrease in growth and plant height in this intercropping pattern. Agegnehu et al. (2006) also reported that, in barley-faba bean intercropping, the faba bean plant height decreased significantly due to interspecific competition. The plant height was higher under CF treatment than those in AMF and AMF+GPB treatments. By increasing the possibility of quick access of fenugreek to a higher nitrogen level, the plant height increased due to increase in plant green area, photosynthetic capacity, and internodes length (López-Bellido et al., 2004).

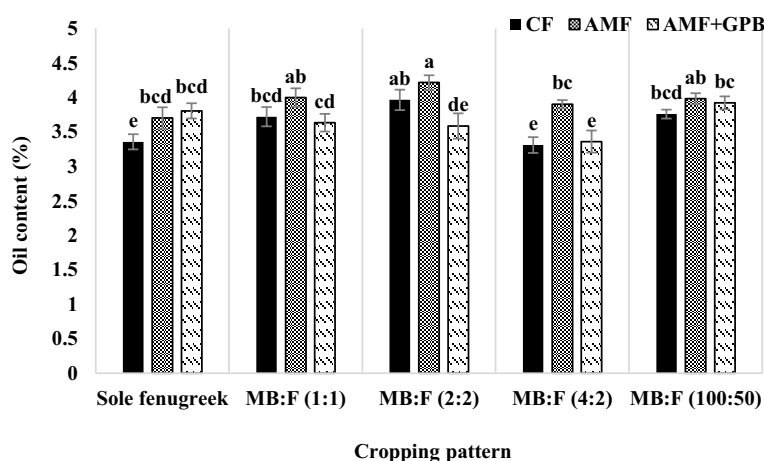


FIGURE 5

Fenugreek oil content as influenced by cropping pattern and fertilizer treatments. Different letters indicate significant differences at $p \leq 0.05$.

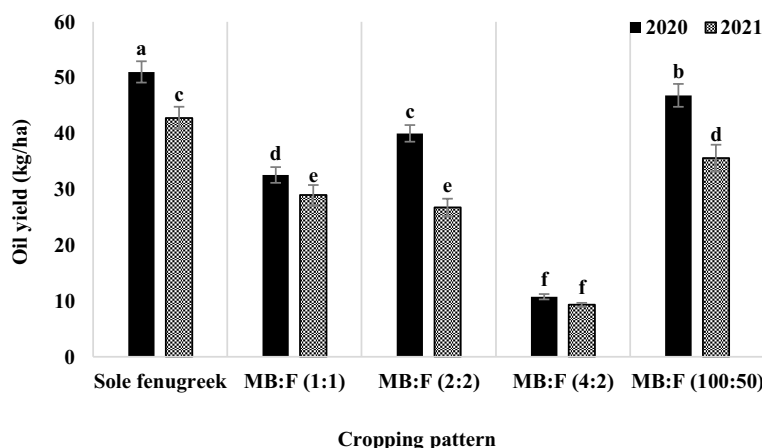


FIGURE 6

Fenugreek oil yield as influenced by year and fertilizer treatments. Different letters indicate significant differences at $p \leq 0.05$.

4.2 Chlorophyll content index (SPAD)

Presumably, in MB:F (2:2) intercropping, the chlorophyll content index in leaves of fenugreek increased in high density and shading conditions of MB to absorb more light and produce photoassimilate (Agegnehu et al., 2006; Singh et al., 2013). The SPAD values of plants treated with AMF+GPB and CF were not significantly different. The effect of biofertilizers on increasing the amount of leaf chlorophyll content is related to better and more plant access to nutrients, such as potassium and nitrogen, provides chlorophyll precursors and increases protein and amino acids as the main precursors of chloroplast structure and activity (Rosas et al., 2006). Huang et al. (2004) also reported that nitrogen plays an essential role in the structure of photosynthetic pigments, including chlorophyll, and it is obvious that the amount of chlorophyll content index will improve with the use of chemical and biofertilizers which increase the N availability.

4.3 Seed yield

All cropping patterns produced higher seed yields in 2020 than those in 2021. The higher precipitation in 2020 growth season could be the main reason for increase in seed yields of all cropping patterns in 2020. Saseendran et al. (2015) reported that climatic variables (mainly precipitation) can have an intensifying effect on crop yield. Similar result is reported by Vafadar-Yengeje et al. (2019) in intercropping of faba bean (*Vicia faba* L.) with MB. After sole fenugreek, the highest seed yield was obtained in MB:F (100:50) that may be attributed to increase in yield in additive intercropping pattern due to higher density of fenugreek compared to replacement intercropping patterns, reduction in weed infestation, proper stratification and better use of environmental resources (Hauggaard-Nielsen et al., 2006; Vrignon-Brenas et al., 2016). Also, more soil coverage in additive intercropping patterns could increase water use efficiency by reducing the evaporation in

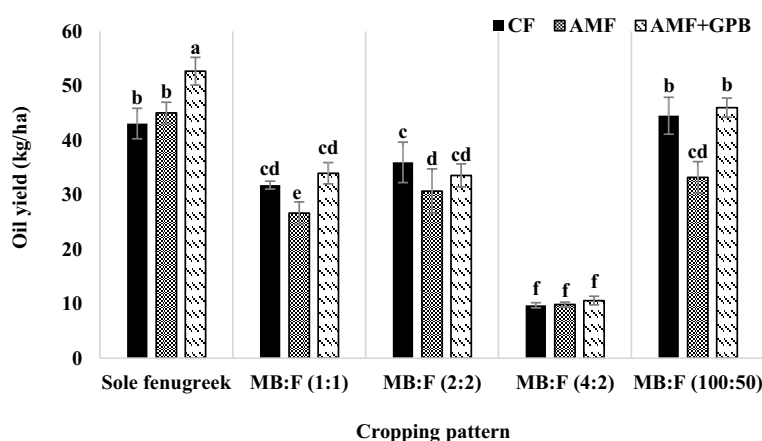


FIGURE 7

Fenugreek oil yield as influenced by cropping pattern and fertilizer treatments. Different letters indicate significant differences at $p \leq 0.05$.

TABLE 5 Proportion of fenugreek oil constituents under different cropping patterns and fertilizer treatments (an average over the two years).

Compounds	RI ^a	Sole fenugreek			MB:F (1:1)			MB:F (2:2)			MB:F (4:2)			MB:F (100:50)		
		CF	AMF	AMF +GPB	CF	AMF	AMF +GPB	CF	AMF	AMF +GPB	CF	AMF	AMF +GPB	CF	AMF	AMF +GPB
Myristic acid	1779	0.15	0.4	0.2	0.15	0.07	0.23	0.2	0.08	0.06	0.02	0.09	0.23	0.17	0.11	0.34
Palmitic acid	1984	11.89	14.12	10.99	8.56	9.98	9.21	11.02	10.26	9.23	8.57	7.65	8.25	8.83	10.32	9.45
Linoleic acid	2112	31.21	31.96	33.67	36.21	36.11	37.01	33.98	35.21	35.98	37.91	38.95	39.21	35.81	36.45	36.41
Linolenic acid	2123	17.65	17.21	17.9	19.05	17.88	17.5	17.98	19.65	20.14	20.14	18.95	21.31	19.85	19.88	19.5
Oleic acid	2141	20.87	20.19	19.95	20.41	20.65	21.1	21.7	21.98	23.65	19.88	21.05	20.56	19.43	19.11	18.79
Stearic acid	2174	11.87	13.2	10.31	9.01	8.56	7.11	10.35	7.65	8.02	7.56	8.52	7.25	8.91	7.46	8.04
Arachidic acid	2366	0.38	0.13	0.19	0.11	0.08	0.39	0.33	0.02	0.06	0.09	0.02	0.05	0.72	0.06	0.53
Behenic acid	2526	0.11	0.08	0.03	0.06	0.03	0.05	0.03	0.07	0.02	0.03	0.06	0.02	0.05	0.05	0.07
Total identified (%)		94.13	97.29	93.24	93.56	93.36	92.6	95.59	94.92	97.16	94.2	95.29	96.88	93.77	93.44	93.13

F, fenugreek; MB, Moldavian balm. CF, AMF, and AMF+GPB are 100% chemical fertilizer, arbuscular mycorrhizal fungi, and growth-promoting bacteria, respectively. Bold values means that the amount of these fatty acids in the fenugreek oil composition is significant.

soil moisture evacuation (Iqbal et al., 2017), so the soil moisture is spent on transpiration of crops, photosynthesis, and yield increase. Among the fertilizer treatments, the highest seed yield was obtained in plants received AMF+GPB treatment. Biofertilizers increase seed yield by creating a cycle of nutrients and making them available and by increasing available water and improving plant growth and development conditions (Grageda-Cabrera et al., 2018). Alizadeh et al. (2019) also reported that, in intercropping of linseed (*Linum usitatissimum* L.) and faba bean, the combined application of PGPR and mycorrhizal fungi, increased the seed yield of both crops. In fact, mycorrhizal symbiosis causes the osmotic regulation of the host plant and increases the contact of the root with soil particles, and then it increases soil nutrients and solubilizes soil minerals due to an increase of microbial activities and lead to improve in absorption of micro- and macro-elements by roots (Kothe and Turnau, 2018). Additionally it has a positive effect on symbiosis of plant with *Rhizobium* (Cardoso and Kuyper, 2006), which in this case can be expected to increase the yield of host plant (fenugreek).

4.4 Oil content (%)

The highest oil content of fenugreek was recorded in MB:F (2:2) intercropping, which could be due to increase in plant’s ability to use environmental resources in this cropping pattern (Agegnehu et al., 2006). The interaction effect of cropping pattern × fertilizer treatment showed that, in all cropping patterns, fenugreek plants treated with AMF had the highest oil content. In general, mycorrhizal fungi improve the plant–soil association by forming hyphae around the plant root, increase the absorption of nutrients such as nitrogen and phosphorus, and consequently improve the fatty acids biosynthesis and oil content (Chen et al., 2017; Fatiha, 2019). It was also observed that the plants treated with CF fertilizer had lower oil content than those in AMF and AMF+GPB. Beaudette et al. (2010) also reported that the use of nitrogen fertilizer in tree-based intercropping system, reduced the oil content of canola (*Brassica napus* L.). The decrease in oil content with the use of CFs has been reported to be related to the inverse relationship between oil content and protein content, in such a way that with increase of nitrogen, the potential production of hydrocarbon substances is reduced and a greater proportion of photosynthetic substances is allocated to the protein synthesis, and as a result, the amount of seed oil decreased (Khan et al., 2002).

4.5 Oil yield

The fenugreek seed yields in 2020 were higher than those in 2021, while the seed oil contents in 2020 were lower. Considering the high correlation between seed yield and oil yield, the fenugreek oil yields in 2020 were higher than those in 2021. The highest seed oil yield was produced in sole fenugreek, since the oil yield is a function of seed yield and oil content. In replacement intercropping patterns, the density of fenugreek is reduced compared to sole cropping; therefore, a decrease in seed yield and consequently in oil yield is expected (Yan et al., 2014). Because of higher seed yield in

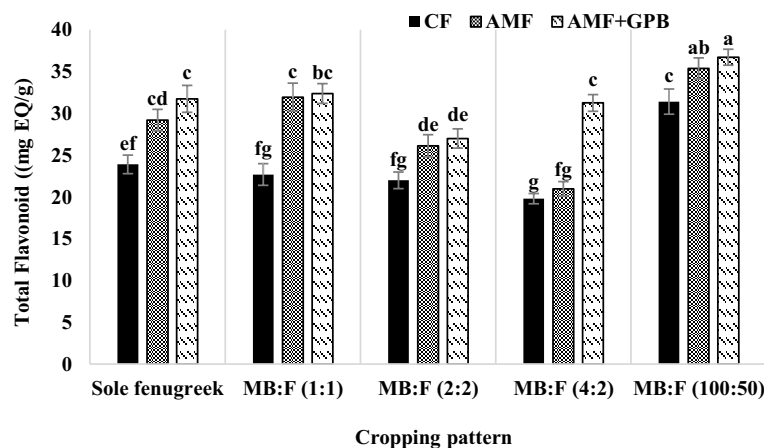


FIGURE 8

Total flavonoid content of fenugreek seeds as influenced by cropping pattern and fertilizer treatments. Different letters indicate significant differences at $p \leq 0.05$.

plants treated with AMF + PGPB and CF, the oil yields in these treatments were also higher. Alizadeh et al. (2019) also reported that the use of biofertilizers, increased the linseed oil yield in intercropping with faba bean. Although, the plants received CF had the lowest oil content among the fertilizer treatments but, in intercropping patterns, the oil yields under CF treatments were not significantly different with those under AMF+GPB. Khan et al. (2018) also reported that using the CFs containing nitrogen had a negative and significant effect on oil content, but due to the positive effect on seed yield, it ultimately increased oil yield.

4.6 Oil composition (GC–MS)

Oil quality depends on the fatty acids composition and the ratio of unsaturated to saturated fatty acids (Fotahi Chiyaneh et al., 2022). It was found that fenugreek seed oil is a rich source of unsaturated fatty acids such as linoleic acid, oleic acid and linolenic

acid that are among the essential fatty acids with beneficial effects on human health (Calder, 2015). Our results are in agreement with the report of Ciftci et al. (2011), Ali et al. (2012), Al-Jasass and Al-Jasser (2012) and Sulieman et al. (2008), as they reported that the unsaturated fatty acids (linoleic acid, oleic acid and linolenic acid) make up most of the fatty acids of fenugreek seed oil. The oil content and fatty acids composition are influenced by factors such as genotype, planting date, soil fertility, planting density, and cropping pattern (Sabzalian et al., 2008). The unsaturated fatty acids contents in intercropping patterns were higher than sole fenugreek and the saturated fatty acids (palmitic acid, stearic acid, etc.) contents in sole fenugreek were higher than all intercropping patterns. It could be concluded that the intercropping patterns improved the environmental conditions for the synthesis of unsaturated fatty acid in fenugreek by increasing the nutrients availability (Gitari et al., 2018; Fotahi Chiyaneh et al., 2022). In most of the intercropping patterns, the contents of unsaturated fatty acids in plants treated with AMF+GPB were higher than those of

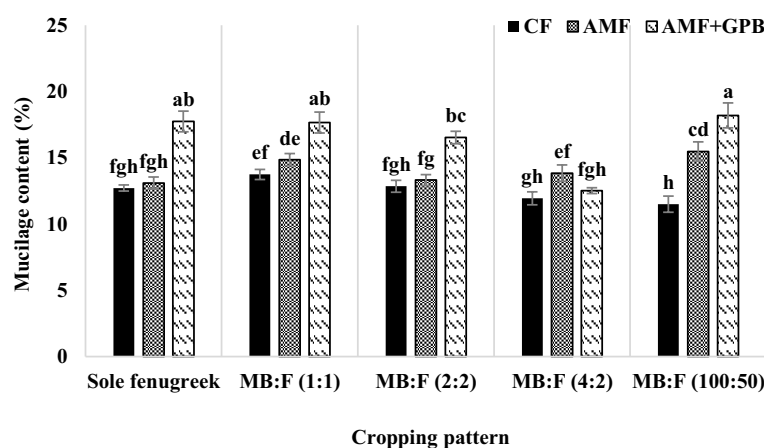


FIGURE 9

Mucilage content of fenugreek seed as influenced by cropping pattern and fertilizer treatments. Different letters indicate significant differences at $p \leq 0.05$.

TABLE 6 Land equivalent ratio (LER) values at different intercropping patterns and fertilizer treatments in 2020 and 2021.

Intercropping pattern	Fertilizer treatment		2020			2021	
		LER _F	LER _{MB}	LER _T	LER _F	LER _{MB}	LER _T
MB:F (1:1)	CF	0.58	0.54	1.12	0.75	0.44	1.19
	AMF	0.58	0.65	1.23	0.58	0.67	1.24
	AMF+GPB	0.68	0.56	1.24	0.71	0.66	1.37
MB:F (2:2)	CF	0.72	0.56	1.28	0.66	0.59	1.25
	AMF	0.65	0.51	1.16	0.55	0.58	1.13
	AMF+GPB	0.75	0.56	1.31	0.67	0.58	1.25
MB:F (2:4)	CF	0.21	0.87	1.08	0.25	0.84	1.09
	AMF	0.20	0.81	1.00	0.22	0.78	1.01
	AMF+GPB	0.24	0.77	1.02	0.24	0.81	1.05
MB:F (100:50)	CF	0.93	0.65	1.58	0.98	0.72	1.70
	AMF	0.74	0.75	1.49	0.69	0.63	1.32
	AMF+GPB	0.83	0.72	1.55	0.87	0.76	1.63

F, fenugreek; MB, Moldavian balm; LER_{MB}, LER of Moldavian balm; LER_F, LER of fenugreek; LER_T, total LER. CF, AMF, and AMF+GPB are 100% chemical fertilizers, arbuscular mycorrhizal fungi, and arbuscular mycorrhizal fungi+ growth promoting bacteria, respectively.

CF treatment, while the contents of saturated fatty acids were higher in plants received CF and AMF treatments. In previous studies, the effectiveness of biofertilizers on increasing the quality of safflower (*Carthamus tinctorius* L.) oil in intercropping with faba bean (*Vicia faba* L.) (Saeidi et al., 2018), olive (*Olea europaea* L.) oil in intercropping with legumes (Chehab et al., 2019) and black cumin (*Nigella sativa* L.) oil in intercropping with fenugreek (Rezaei-Chiyaneh et al., 2021) has been reported. It was found that the use of CFs caused a decrease in unsaturated fatty acids and oil quality in oilseeds (Sharma, 2005). The use of biofertilizers improves access to nutrients by improving soil microbial activity and root development (Dawood et al., 2019), and production of fatty acid precursor compounds, which leads to an increase in unsaturated fatty acids contents and oil composition (Shu-tian et al., 2018). When nitrogen is available in a sufficient amount to the plant, leaf senescence occurs later and the plant can remobilization photoassimilate to its leaves for a longer time (Diacono et al., 2013); therefore, plant GPB cause the continuation of plant growth and improve the oil quality by supplying the nitrogen needed by the plant in reproductive stages. In this study, AMF+GPB treatment improved the quality of fenugreek oil (increased the contents of unsaturated fatty acids) due to the synergistic effects of GPB in nitrogen absorption and mycorrhizal fungi in providing suitable conditions for absorption of water, micro- and macro-elements.

4.7 Total flavonoid and anthocyanin

Flavonoid compounds are the result of the phenylpropanoid pathway, and the phenylalanine ammonia lyase (PAL) enzyme is the initiator of this pathway, which plays an essential role in formation of phenolic compounds and is raised as one of the

indicators sensitive to environmental changes such as planting density and climate changes (light, temperature, humidity) (Vogt, 2010; Miranda et al., 2012). Therefore, it seems that the higher air temperature and lower precipitation in 2021 were effective in synthesis of the mentioned enzyme and in this way increased the synthesis of flavonoids through phenylpropanoid pathway. Also, the increase in biosynthesis of flavonoids in intercropping patterns [especially in MB:F (100:50)] may be due to activation of the plant's defense strategy against competitive stress (Winyard et al., 2005). Dehghani Mashkani et al. (2011) also reported that biofertilizer treatments caused a significant increase in flavonoid content in chamomile (*Matricaria recutita* L.). Since flavonoids and other secondary metabolites are by-products of photosynthesis, application of biofertilizers increased their synthesis by improving the leaf area and nutrients availability (Mona and Khalil, 2006). The lower contents of total flavonoid and anthocyanin in plants treated with CF could be explained by protein competition model or growth differentiation balance. According to this theory, when the biomass increases in response to more availability of nitrogen, the concentration of phenolic compounds decreases, because the increase in plant's need for protein for growth reduces the phenolic compounds, as well as biomass accumulation dilutes the concentration of phenolic compounds (Ibrahim et al., 2010).

4.8 Mucilage content (%)

Mucilage compounds are insoluble hydrocarbons in fenugreek seed and part of plant secondary metabolites (Wu et al., 2009). Increasing the mucilage content in 2021 could be attributed to lower precipitating and higher temperatures. Also, higher mucilage contents in MB:F (1:1) and (100:50) intercropping patterns may be due to the increase in interspecific competition (Miranda et al.,

2012). The higher mucilage contents in biofertilizer treatments (AMF+GPB and AMF) at all cropping patterns indicates that mucilaginous compounds as one of the secondary metabolites can be influenced by increasing the availability of water and nutrients for plant caused by inoculation of biofertilizers (Yousefi et al., 2011).

4.9 Land equivalent ratio of intercropping

The partial LERs for fenugreek (LER_F) were higher than those of MB (LER_{MB}), which indicates that intercropping has a positive effect on fenugreek than MB. Monti et al. (2016) reported that the increase in partial LER higher than 0.5 depends on complementary degree of the intercropping components. Also, LER_T higher than 1.0 obtained in all intercropping patterns and fertilizer treatments indicate that intercropping is more advantage than sole cropping (Amini et al., 2020). The superiority in intercropping is due to different morphological and growth properties and the tendency of intercropping components to make optimum use of resources such as soil moisture, light and nutrient elements, and there are differences in root structure, distribution of the canopy cover, and nutritional needs of plants in the intercropping (Hauggaard-Nielsen et al., 2008). The role of morphological differences in achieving higher LER_s , have been reported in intercropping of soybean–sugar cane (Morsy et al., 2017), wheat–fenugreek (Wasaya et al., 2013), maize–pea (Mao et al., 2012), and faba bean–MB (Vafadar-Yengeje et al., 2019). The results of some studies have also shown that, when the legume species beside the other species are planted as an intercropping, due to the complementary effect, nitrogen stabilization is stimulated, which increases the growth and yield of the legume species due to the increase in the number of active nodes (Zhao et al., 2017). Although the presence of species together increases competitiveness to absorb environmental resources, if one species has nitrogen fixation ability, competitive pressure will be reduced, because the legume species will have less competition with other species in nitrogen absorption as one of the main and most restrictive factors (Karpenstein-Machan and Stuelpnagel, 2000).

5 Conclusions

Among the intercropping patterns, the highest seed and oil yield of fenugreek were observed in MB:F (100:50) pattern and the lowest ones in MB:F (4:2). In all intercropping patterns, the oil yields in plants received AMF+GPB and CF were not significantly different. The GC–MS analysis of fenugreek oil indicated that the contents of unsaturated fatty acids (linoleic and linolenic acids) increased in intercropping patterns compared with sole cropping. Also, in sole cropping of fenugreek and all intercropping patterns, the linoleic acid content increased in plants treated with AMF+GPB, compared with that in CF. The anthocyanin, total flavonoid, and mucilage contents were

improved in plants under MB:F (100:50) intercropping pattern and AMF+GPB treatment. The highest LER_T values were observed in MB:F (100:50) intercropping pattern ($CF = 1.70$, $AMF + GPB = 1.63$). Generally, we can conclude that, in sustainable production systems, the fenugreek sole cropping and CF application could be replaced with additive intercropping of MB:F (100:50) and inoculation with AMF + GPB (AMF+GPB). These strategies will help the growers to improve the fenugreek oil yield a composition and reduce the harmful effects of CFs on agro-ecosystems.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

ZA: Formal analysis, Investigation, Methodology, Data curation, Software, Writing – original draft. RA: Formal analysis, Investigation, Methodology, Conceptualization, Project administration, Supervision, Validation, Writing – review & editing. AD: Conceptualization, Investigation, Methodology, Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Acknowledgments

We thank the University of Tabriz for their technical support.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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OPEN ACCESS

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RECEIVED 19 December 2023

ACCEPTED 29 May 2024

PUBLISHED 09 August 2024

CITATION

Wang S, Fan T, Zhao G, Ma M, Lei K, Li S,
Cheng W, Dang Y, Wang L, Zhang J,
Zhou G, Li X and Ni S (2024) Effect of
integrated fertilizer and plant density
management on yield, root characteristic
and photosynthetic parameters in
maize on the semiarid Loess Plateau.
Front. Agron. 6:1358127.
doi: 10.3389/fagro.2024.1358127

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Effect of integrated fertilizer and plant density management on yield, root characteristic and photosynthetic parameters in maize on the semiarid Loess Plateau

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Introduction: Improving photosynthetic use efficiency in dryland agroecosystems to sustain high agricultural yields is a key responsibility for ensuring food security.

Methods: This study was conducted in the regions on the semiarid Loess Plateau of China during 2018–2020. Dryland maize of Xianyu 335 comprised four modes: basic yield input (CK, plastic film mulching, 37500 plant.ha⁻¹ of plant density and unfertilized), farmer input (FP, plastic film mulching, 45000 plant.ha⁻¹ of plant density and inorganic nitrogen(N) and phosphate(P) fertilizer were 150kg.ha⁻¹ and 90kg.ha⁻¹), high yield and high-efficiency input (HH, full plastic-film mulching on double furrow, 67500 plant.ha⁻¹ of plant density and N, P and organic manure(M) fertilizer were 230kg.ha⁻¹, 140kg.ha⁻¹ and 1500kg.ha⁻¹), and super high yield input (SH, full plastic-film mulching on double furrow, 9000 plant.ha⁻¹ of plant density and N, P and organic M fertilizer were 300kg.ha⁻¹, 180kg.ha⁻¹ and 7500kg.ha⁻¹). The effects of different cultivation modes on yield, WUE, net photosynthetic rate(P_n), leaf area index(LAI), chlorophyll index(SPAD value) and root index were studied.

Results: The results showed that the value average of yield and WUE for CK were 7790kg and 17480kg.ha⁻¹ in three years. SH, HH and FP cultivation modes of yield and WUE was significant higher compared with CK cultivation mode ($P < 0.05$). SH, HH and FP cultivation modes of yield and WUE increased by 34.01%, 48.68%, 56.39% and 34.34%, 47.99%, 57.99%, compared than CK cultivation mode. These differences were observed during the seedling stage, jointing stage, silking stage and filling stage. Year to year variation in performance of applied treatment, this improved in CK cultivation mode significantly enhanced SPAD value, P_n , LAI and

the root index than SH, HH and FP cultivation modes. The yield exhibited a positive correlation with the WUE, SPAD value, P_n , LAI. The SH cultivation mode was the highest yields.

Discussion: The results indicated that maize yield and WUE could be increased through integrating and optimizing cultivation techniques in maize production on the semiarid western Loess Plateau of China. The SH cultivation mode was the highest yields. The primary factor contributing to the increase in yield and WUE of maize due to increased density, increased fertilizer and covering measures is the augmentation of P_n , LAI, SPAD value, and root index.

KEYWORDS

maize, yield, water use efficiency, photosynthetic parameters, root index, semiarid Loess Plateau

1 Introduction

The largest cultivation area and yield of all crops worldwide is produced by maize (*Zea mays* L.) (Zhao, 2022). Globally, more than 197 million hectares of maize-producing land are cultivate worldwide, over yielding 1.13 billion tons of maize (Queenta et al., 2022). A total area of 42.42 million ha of maize are cultivated in China with yield of approximately 259.23 million tons per year (Ramadan et al., 2021). Maize has been widely cultivated in recent years on the semiarid western Loess Plateau of China (Xu and Zhang, 2017). The progression of urbanization has resulted in a dearth of cultivable land and water resources, while the widespread application of fertilizers and pesticides has contributed to the deterioration of soil quality and a reduction in grain output. Concurrently, population growth has engendered anthropogenic environmental degradation, while climate change has given rise to extreme temperatures, including both frigid winters and scorching summers. Moreover, the occurrence of late spring frost has engendered a trend of decelerating or even stagnant growth rates in maize production across global nations. The growth rate of maize production can't catch up with the demand of population, energy and feed, and the global food production and security are facing great challenges (Barret, 2010). A previous study on maize high yields showed that increased yields required adequate water and fertilizer, high yielding varieties and tolerant varieties, high planting densities, and reasonable cultivation measures (Wang et al., 2020). Therefore, maize variety and innovate cultivation techniques have become effective ways to increase the maize yield per unit area in the context of the rigid demand for maize yield, the reduction of cultivated land and water shortage in China (Zi et al., 2022).

There exist several strategies for enhancing maize yields, including denser planting, precise management of water and fertilizer, and adoption of specific planting techniques (Wu et al., 2015; Raza et al., 2021). WUE increased linearly as yield increased on the Loess Plateau under the different mulching and tillage

practices. As the population reaches a certain threshold, inter-individual competition intensifies, leading to the development of a constrained canopy environment that hampers the attainment of maize's yield potential. The selection of appropriate varieties, consideration of climatic conditions, and implementation of suitable cropping practices all play a crucial role in influencing light availability, field microclimate, and other factors that collectively enhance the photosynthetic performance of the maize population, thereby augmenting its yield (Xu et al., 2020). The growth and distribution of roots within the soil profile are significantly influenced by both soil moisture levels and genetic factors. Research has shown that regular irrigation promotes root development in the upper layers of soil, while dry conditions encourage deeper root growth. Additionally, soil management techniques such as tillage, sowing, and the incorporation of organic matter into the soil have been found to enhance root proliferation, as indicated by an increase in root length density (Benjamin and Nielsen, 2006. Schulze et al., 1996. Aggarwal and Sharma, 2002. Aggarwal and Goswami, 2003). The effect of different cultivation patterns from different root length and root diameter. Plant density at the right level contributes to an increased stand LAI, improved solar radiation utilization, and improved maize yields and WUE (Jia et al., 2018). The Leaf SPAD value was utilized as a metric for assessing leaf chlorophyll content and exhibited a strong correlation with leaf photosynthetic characteristics. The SPAD readings of rice leaves exhibited variability across three distinct stages of leaf development: initial growth, peak functionality, and senescence (Xu et al., 2019). SPAD readings and normalized SPAD values are positively correlated with maize yield (Yuan et al., 2016). Light affects many aspects of plant growth and development, not only supplies an energy source for photosynthesis, but also acts regulatory signal that controls plant development. The amount of light exposure can vary significantly due to differences in the population structure of the plants, resulting in significant differences in the photosynthetic efficiency of the

leaves. P_n is directly related the amount of organic matter accumulated, thus impact yield (Barrret, 2010). The root system of maize plays a vital role in the processes of absorption, synthesis, fixation, and support (Qi et al., 2014). The growth and development of this system directly influence the maize plant's capacity to absorb and utilize water and nutrients, ultimately impacting the production of plant dry matter and maize yield formation (Qi et al., 2012). Through the analysis of yield and LAI of spring maize in different cultivation modes, the LAI of the high-density, organic fertilizer and nitrogen fertilizer transport tillage two-by-two (T4) cultivation mode was significantly higher than that of the low-density, no-fertilizer rotary-tillage equidistant row spacing (T1) cultivation mode (Wang et al., 2020). Currently, a significant approach to enhancing maize yield and optimizing resource utilization is through the regulation of the maize root system's growth, which promotes the uptake and efficient utilization of water and nutrient. It has been demonstrated that rational fertilization practices can effectively modulate root growth (Ren et al., 2017). Additionally, Liu Shengqun et al. discovered a significant positive relationship between root dry weight and variables including green leaf area, above-ground dry weight, and seed yield (Liu et al., 2007).

Previous studies have been limited to maize population structure and photosynthetic performance, and there have been fewer studies on the effects of different cultivation modes on spring maize yield and root development the Northern Loess Plateau. Hence, this experimental site has been established to explore various cultivation modes, primarily focusing on increasing density and optimizing fertilizers and mulching, based on an investigation of the prevailing planting practices among farmers in the Northern region. The objective of this study is to analyze the impact of various cultivation modes on the development of spring maize yields and the efficiency of photosynthetic utilization. This analysis is conducted through a comparative examination of P_n , LAI, SPAD value, and changes in root index on the Northern Loess Plateau. The findings of this research aim to offer theoretical foundations and technical guidance for the enhancement of high-yield maize cultivation.

2 Materials and methods

2.1 Experimental site and design

The experiment was conducted in Shangxiao Town, Zhenyuan County, Qingyang City (107.2° E, 35°68'N, 1295m above sea level) situated in Gansu Province, China, during the period of 2018 to 2020. The soil types observed were dark loessial soil. The average annual precipitation recorded was 510mm during the year 2018. Based on the data depicted in Figure 1, the annual precipitation was documented as 646.0mm. Throughout the growth phase, Zhenyuan experienced a rainfall of 575.8mm in 2019. Zhenyuan encountered rainfall quantities of 587.5mm in 2020. It is noteworthy that the precipitation in 2018 exceeded that of both 2019 and 2020. Additionally, the average temperature during the growth period for corn cultivation, which spans from April to September, exhibited a gradual decline from 2018 to 2020. Specifically, the average temperatures were recorded as 20.17 °C, 19.45 °C, and 19.43°C for the respective years. The chemical properties of the test site soil are presented in Table 1 (Zhang et al., 2023).

These treatments, namely SH, HH, FP and CK differed in terms of cover methods, planting densities, and fertilizer management. With the exception of the CK treatment, all other treatments received uniform application of N, P, K, and organic fertilizer prior to land preparation and film covering. No additional fertilizer was introduced throughout the entire growth period. Field management adhered to practices employed in high-yield fields. Sowing was conducted using the full film double ridge furrow method, employing a polyethylene membrane with a width of 0.7mm and thickness of 0.01mm. The experimental plot consisted of a row measuring 5m in length, with line spacing of 0.75m and plant spacing of 0.30m. The planting density was 75000 plants per hectare. All seedlings were managed under the general field management methods. Four treatments were applied, and each was repeated 3 times. Effective accumulated temperature, growth length and photosynthetically active radiation can be found in Table 2. Detailed information regarding each treatment can be

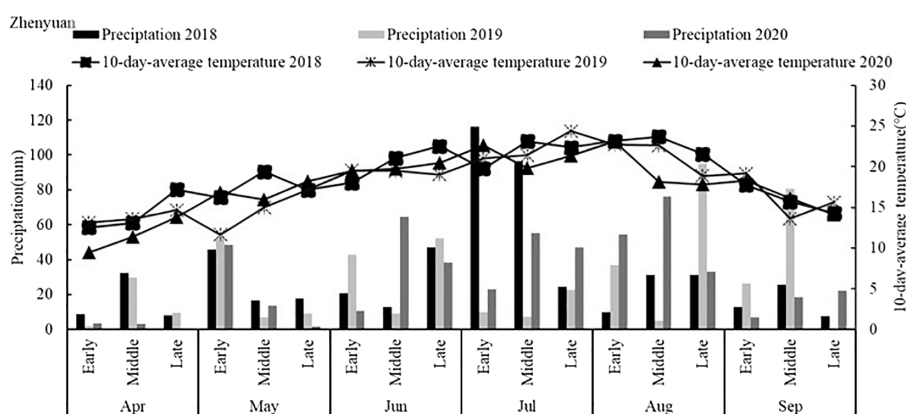


FIGURE 1
Precipitation and temperature during maize growth season in 2018–2020.

TABLE 1 Chemical properties of 0–20 cm soil.

Class	Content(g.kg ⁻¹)
Total nitrogen	0.97
Available nitrogen	0.29
Available phosphorus	0.95
Available potassium	14.02
Organic matter	21.58

found in Table 3. Each year, all treatments received a basal application in the form of triple superphosphate (16% P₂O₅), N (100% N) and M (100% M). N and P were obtained STANLEY. M of (N + P₂O₅ + K₂O ≥ 5%; organic content ≥ 45%) was obtained HONGYUAN.

No potassium fertilizer(K) was added. The experiment involved testing four treatments within the same plot, all of which had uniform fertility. The experiment had four treatments each consisted three replications. The experiment encompassed a substantial area of 225m². The experiment utilized the spring maize variety of Xianyu 335, which exhibited robust stress resistance and consistent yield stability. Maize was planted in late April and harvested in late September. No irrigation throughout the entire growth period of maize.

2.2 Sampling and measurements

2.2.1 Measurement of yield

Four maize rows in each treatment plot were selected as consistent growth. A total of three 5.5m² corn ears were collected from each treatment and threshed to calculate the yield in the maturity stage.

2.2.2 Measurement of WUE

WUE = Economic yield of crops(kg.ha⁻¹)/Total water consumption during the crop growth period (mm).

2.2.3 Measurement of LAI

Thirty leaves near the functional leaves were randomly selected for each treatment, and were measured with a crop leaf morphometer (TPYX-A, Hangzhou, China) in the seedling stage, jointing stage, silking stage and filling stage, and the data were recorded, and the process was carried out for five times in total.

TABLE 2 Effective accumulated temperature, growth length and photosynthetically active radiation.

	2018	2019	2020
Effective accumulated temperature(°C)	2508.5	2650.73	2713.78
Growth length(d)	1008.5	1300.73	1323.78
Photosynthetically active radiation	1473.6	1170.05	1123.78

TABLE 3 Mulching, planting density, and fertilizer management of different cultivation modes treatment.

Treatment	Mulching	Plant density (plant.ha ⁻¹)	Fertilizers (kg.ha ⁻¹)
CK	Plastic film mulching	37500	N=0, P ₂ O ₅ = 0, M=0
FP	Plastic film mulching	45000	N=150, P ₂ O ₅ = 90
HH	Full plastic-film mulching on double furrow	67500	N=230, P ₂ O ₅ = 140, M=1500
SH	Full plastic-film mulching on double furrow	90000	N=300, P ₂ O ₅ = 180, M=7500

CK, base level; FP, farmer's level; HH, high yield and high efficiency; SH, super high yield. N, nitrogenous fertilizer, P₂O₅, phosphate fertilizer, M, commercial organic fertilizer.

2.2.4 Measurement of P_n

The experiment was conducted on a sunny day and measurements were taken at 10:00 to 11:00 am. P_n was measured in maize during the field trial in 2018–2020 at the Zhenyuan site. Measurements were made on healthy and fully expanded leaves of randomly chosen plants at different growth stages (seedling stage, jointing stage, silking stage and filling stage). The P_n was assessed with a portable photosynthesis system (LI-6800, LI-COR, NE, USA). Five readings were repeated for each leaf. When determining the indexes, it is necessary to avoid the main leaf veins and record the data after the system is stabilized. The light intensity and air temperature were determined in a natural environment, and the CO₂ concentration was set at 400 μmol mol⁻¹, and the CO₂ concentrations in the sample and reference chambers were matched during the warm-up period of the instrument.

2.2.5 Measurement of SPAD values

The determination of leaf chlorophyll content was carried out in the morning from 9:00 to 11:00 a.m. For each treatment, 15 leaves were randomly selected near the functional leaves of plants with labels, and the SPAD values of the leaves were measured using a hand-held Top TYS-B Portable SPAD Chlorophyll Content Detector (TYS-B, Zhejiang, China), and the SPAD values of each leaf were measured three times and recorded randomly chosen plants at different growth stages (seedling stage, jointing stage, silking stage and filling stage). The values were recorded.

2.2.6 Measurement of root index

Roots of maize seedlings were taken and its were washed with water and the residual water on the surface was blotted with absorbent paper. The roots of maize plants were scanned with a root scanner (TD4800, Canada) and the pictures were saved, and the pictures of the roots were batch analyzed with the software Win RHIZO (Pro 2.0 Version 2005; Regent Instruments, Quebec, QC, Canada), which in turn yielded the length of the root system, surface area, number of root tips and volume, and other relevant indicators in the seedling stage.

2.3 Statistical analysis

The data were analyzed using SPSS software (version 21.0: IBM Corp., Armonk, NY, USA). The data was analyzed using a two-way analysis of variance with Duncan's multiple-range test. A value of $p < 0.05$ was considered statistically significant, and a value of $p < 0.01$ was considered very significant. Origin 2021 (Origin Lab, Massachusetts) software was used to draw graphs. The tables and graphics were created using Excel 2019.

3 Results

3.1 Effect of different cultivation modes on maize yield

The yield was significantly different from different cultivation modes ($P < 0.05$). The yield order of the different cultivation modes was $SH > HH > FP > CK$, and the data of the 3-year field trial showed the same trends (Table 4). Maize yield was significantly higher under SH, HH and FP cultivation modes as compared to the CK in 3-year ($P < 0.05$). In the 3-year trial, the average yield of SH, HH and FP cultivation modes increased by 34.01%, 48.68% and 56.39% compared with CK cultivation mode. SH and HH cultivation modes increased by 22.24% and 33.92% compared with FP cultivation mode. SH cultivation mode increased by 15.01% compared with HH cultivation mode. The average yield of 2018 increased by 11.05% and 24.94%, compared with 2019 and 2020, respectively. The CK cultivation mode had the largest increase in yield in 2020 compared with 2019 and 2020 yields, increasing by 38.37% and 25.90%. The yield in 2018 of the SH, HH, FP, CK cultivation modes 38.41%, 8.56%, 4.29%, 4.88% and 25.91%, 20.78%, 20.42%, 30.85% was increase as in 2019 and 2020.

3.2 Effect of different cultivation modes on WUE

The WUE order of different cultivation modes was $SH > HH > FP > CK$, the data of the 3-year field trial showed the same trends. WUE was significantly higher under SH, HH and FP cultivation modes as compared to the CK in 3-year ($P < 0.05$). In the 3-year trial, SH, HH, and FP cultivation modes of average WUE were 34.34%, 47.19% and 57.99% increase as CK cultivation mode. SH and HH cultivation modes were 19.57% and 36.02% larger than the FP cultivation mode. The average WUE in 2018 20.53% and 16.19% was increase as in 2019 and 2020 (Table 5).

3.3 Effect of different cultivation modes on SPAD

As shown in Figure 2, the SPAD values of maize in seedling stage, jointing stage, silking stage, and filling stage of different

TABLE 4 Yield of maize under different cultivation modes.

Treatment	Yield(t.ha ⁻¹)			Average
	2018	2019	2020	
CK	9.92dA	6.11dB	7.35dB	7.79
FP	13.09cA	11.97cB	10.37cB	11.81
HH	16.55bA	15.84bA	13.17abB	15.19
SH	20.29aA	19.30aA	14.03aB	17.87

CK, base level; FP, farmer's level; HH, high yield and high efficiency; SH, super high yield. Different lowercase letters indicate that the different cultivation is significantly different among materials ($P < 0.05$), different capital letters indicate that the different year is significantly different among materials ($P < 0.05$).

cultivation modes during the experimental period showed that $SH > HH > FP > CK$. During the three-year period, the SPAD values of SH, HH and FP cultivation modes was on an average 27.71%, 54.21% and 59.81% in seedling stage (Figure 2A), 28.07%, 40.01% and 55.80% in jointing stage (Figure 2B), 33.21%, 41.05% and 58.16% in silking stage (Figure 2C) and 33.19%, 47.80% and 49.25% in filling stage (Figure 2D) greater than CK cultivation mode. The SPAD values in 2019 and 2020 were lower by 4.53% and 7.23% than in 2018.

3.4 Effect of different cultivation modes on P_n

The P_n of maize in seedling stage, jointing stage, silking stage, and filling stage different significantly among different cultivation modes. The P_n order of the different cultivation modes was $SH > HH > FP > CK$, and the data of the 3-year field trial showed the same trends in different stage. During the three years, the P_n of SH, HH and FP cultivation modes was on an average 15.26%, 18.71% and 29.86% in seedling stage (Figure 3A), 7.54%, 18.46% and 22.89% in jointing stage (Figure 3B), 15.47%, 22.48% and 22.89% in silking stage (Figure 3C) and 25.31%, 29.79% and 35.27% in filling stage (Figure 3D) greater than CK cultivation mode. The average P_n in 2019 were higher by 14.33% and 21.07% than in 2018 and 2020.

TABLE 5 WUE of maize under different cultivation modes.

Treatment	WUE(kg.ha ⁻¹ .mm ⁻¹)			Average
	2018	2019	2020	
CK	18170dA	17570dA	16710dA	17480
FP	24430cB	30710cA	24740cB	26630
HH	29350bB	38360bA	31590abB	33100
SH	35660aC	48750aA	40430aB	41610

CK, base level; FP, farmer's level; HH, high yield and high efficiency; SH, super high yield. Different lowercase letters indicate that the different cultivation is significantly different among materials ($P < 0.05$), different capital letters indicate that the different year is significantly different among materials ($P < 0.05$).

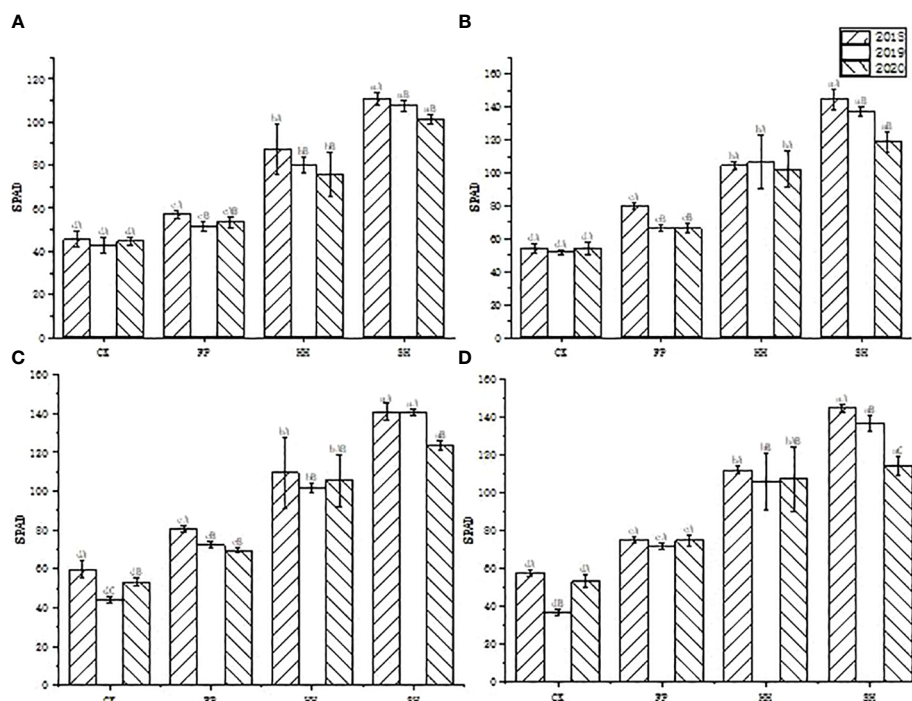


FIGURE 2

SPAD of maize under different cultivation modes. (A) seedling stage, (B) jointing stage, (C) silking stage, (D) filling stage. CK, base level; FP, farmer's level; HH, high yield and high efficiency; SH, super high yield. Different lowercase letters indicate that the different cultivation is significantly different among materials ($P < 0.05$), different capital letters indicate that the different year is significantly different among materials ($P < 0.05$). The error bar represents the standard error of the average of the sample.

3.5 Effect of different cultivation modes on LAI

The LAI of maize in seedling stage, jointing stage, silking stage different significantly among different cultivation modes. The LAI order of the different cultivation modes was $SH > HH > FP > CK$, and the data of the 3-year field trial showed the same trends in different stage. During the three years, the average LAI of SH, HH and FP cultivation modes increased by 59.77%, 77.01% and 82.83% in seedling stage (Figure 4A), 21.91%, 48.44% and 61.37% in jointing stage (Figure 4B), 18.16%, 47.47% and 61.33% in silking stage (Figure 4C) compared with CK cultivation mode. The average LAI in 2020 were higher by 14.33% and 21.07% than in 2019 and 2020.

3.6 Effect of different cultivation mode on root index

The root index of different cultivation modes were different (Figure 5). SH cultivation mode significantly enhances maize root length (Figure 5A), root diameter (Figure 5B), number of root tips (Figure 5C) and root surface (Figure 5D) area with a 71.10%, 10.75%, 61.79% and 51.70% higher root length than that of CK cultivation mode. Root length was higher 0.44% and 8.34% in 2020 than that of 2018 and 2019, root diameter, number of root tips, root surface area were significantly higher 14.69%, 25.87%, 46.06% and 8.92%, 10.45%, 22.18% in 2018 than that of 2019 and 2020.

3.7 Correlation analysis of yield with WUE, P_n , SPAD, LAI and root index

There was a highly significantly positive correlation between yield and WUE, P_n in silking and filling stage, LAI, SPAD value and root index, but was not correlated with P_n in seedling and jointing stage (Table 3). The highly significantly positive correlation was detected between WUE and P_n in silking and filling stage, LAI, SPAD value and root index. Significantly positive relationship between WUE and P_n in seedling and jointing stage.

4 Discussion

4.1 Effect of different cultivation modes on yield and WUE in maize

In Li Shangzhong's study, an analysis was conducted on the maize yield and WUE of various film cover cultivation modes. The findings revealed that the full-film double-row furrow cultivation mode exhibited a significant increase of 21.9% and 31.3% in yield and WUE, when compared with the open field cultivation mode (Li et al., 2020). The cultivation mode had a significant impact on the yield and nutrient use efficiency of dryland spring maize (Zhu, 2009). This trend was similar with the results of Lal and Stewart and Zhong et al. for the same region. However, the WUE-yield

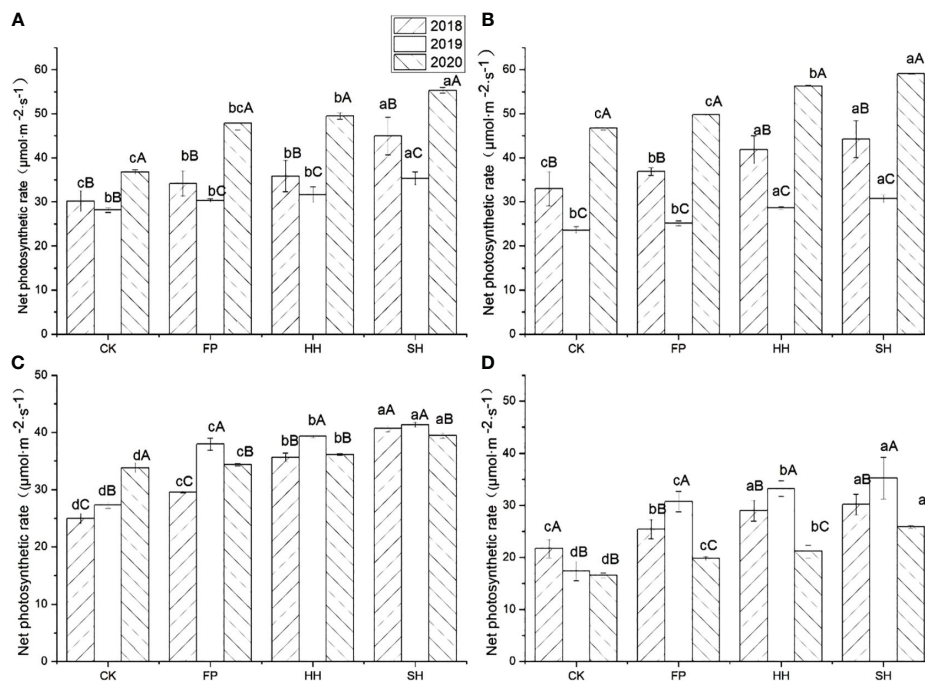


FIGURE 3

P_n of maize under different cultivation modes. (A) seedling stage, (B) jointing stage, (C) silking stage, (D) filling stage. CK, base level; FP, farmer's level; HH, high yield and high efficiency; SH, super high yield. Different lowercase letters indicate that the different cultivation is significantly different among materials ($P < 0.05$), different capital letters indicate that the different year is significantly different among materials ($P < 0.05$). The error bar represents the standard error of the average of the sample.

relationship was quadratic when the full range of yield was considered (Zhong and Shangguan, 2014). The optimization of cultivation modes or the implementation of integrated agronomic measures has been found to have a significant impact on crop yield

improvement (Jin, 2013). Our results demonstrates that the SH, HH modes exhibited superior yields, as evidenced by significantly higher of yield and WUE compared to the FP and CK cultivation modes.

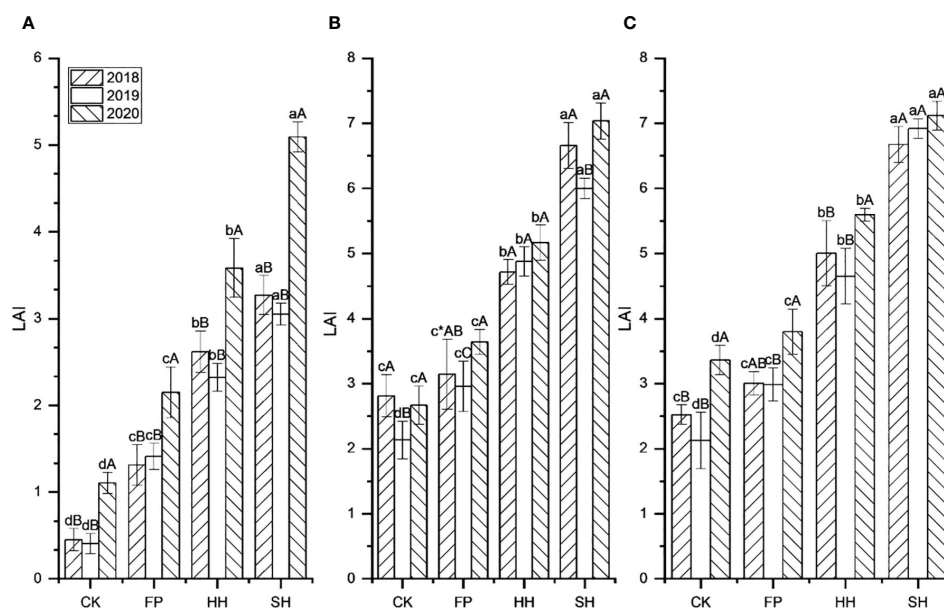


FIGURE 4

LAI of maize under different cultivation modes. (A) seedling stage, (B) jointing stage, (C) silking stage. CK, base level; FP, farmer's level; HH, high yield and high efficiency; SH, super high yield. Different lowercase letters indicate that the different cultivation is significantly different among materials ($P < 0.05$), different capital letters indicate that the different year is significantly different among materials ($P < 0.05$). The error bar represents the standard error of the average of the sample.

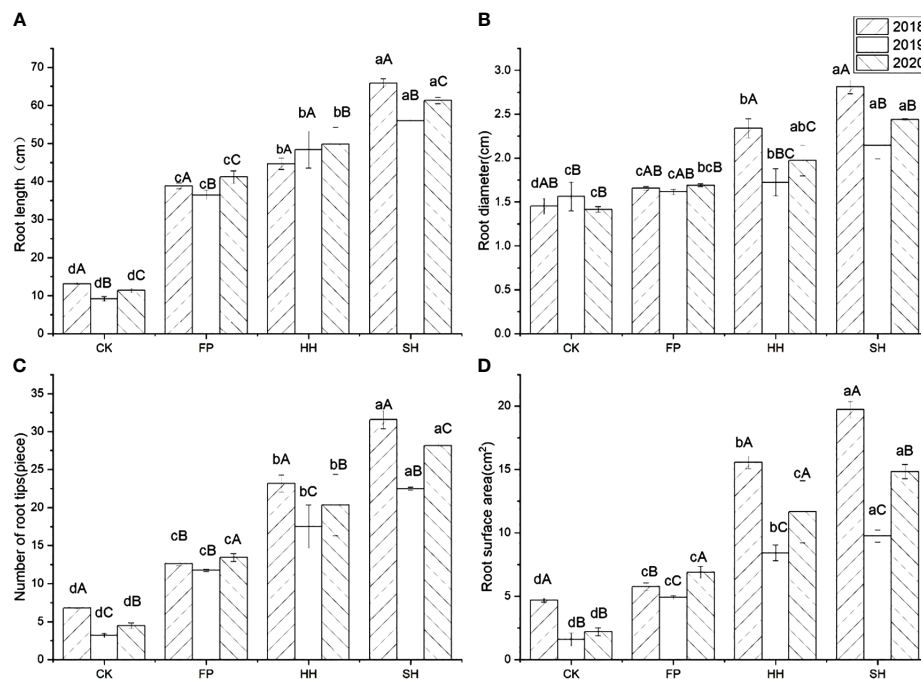


FIGURE 5

Root index of maize under different cultivation modes. (A) root length, (B) root diameter, (C) number of root tips, (D) root surface area. CK, base level; FP, farmer's level; HH, high yield and high efficiency; SH, super high yield. Different lowercase letters indicate that the different cultivation is significantly different among materials ($P < 0.05$), different capital letters indicate that the different year is significantly different among materials ($P < 0.05$). The error bar represents the standard error of the average of the sample.

4.2 Effect of different cultivation modes on photosynthetic parameters in maize

The main place of plant photosynthesis is the leaf, which directly affects plant absorption and light energy utilization. LAI is an important indicator reflecting plant growth, development and light energy utilization, and maize should maintain a high LAI to achieve high yields (Zhang et al., 2011). Liu et al. found that LAI with the A3 treatment increased at the early growth stage (tillering) compared with A0 but decreased in subsequent growth stages and became lower than A0 at maturity. This may be due to the fast consumption of soil N by straw decomposition mediated by microbes and crop growth at early growth stages, resulting in insufficient nutrient supplies for subsequent growth (Liu et al., 2023; Cai et al., 1986). The application of CRF treatment resulted in a significant improvement in leaf chlorophyll content, delayed the reduction in chlorophyll levels in the leaf, enhanced the Leaf Area Index (LAI), and increased the maximum P_n during the pod-filling and mature stages of peanut growth (Liu et al., 2019). Nitrogen fertilizer transport can effectively regulate root growth, SPAD value is the result of the integrated effect of multiple factors (Zhuang et al., 2013). The previous study showed that rational fertilization is conducive to increase the maize LAI, improve the leaf SPAD value, and enhance the P_n of maize after spatulation (Bian et al., 2008). Previous studies on maize yield, LAI and SPAD value under different cultivation modes found that the LAI and SPAD of the super-high yielding cultivation mode increased by 80.03% and 13.73%, compared with than that of the farmer mode in Tibetan areas of the Western Sichuan Plateau. Our results demonstrates that the super-

high yield mode and high yield and efficient cultivation mode exhibited superior yields, as evidenced by significantly higher values of LAI, P_n and SPAD values compared to the FP and CK cultivation modes. LAI, P_n and SPAD values of increase is due to the increase in rain, and the need for later compound synthesis. Furthermore, the SH and HH cultivation modes demonstrated a longer duration period of LAI, P_n and SPAD values.

4.3 Effect of different cultivation modes on root index in maize

The relationship between the size of the crop root system and crop yield is significant. A robust root system plays a crucial role in providing adequate nutrients and water for the growth and development of corn, thereby facilitating the realization of its high yield potential. Researchers have observed a noteworthy positive correlation between indicators such as root dry weight, root length, root surface area, and crop yield (Table 6). The root development of summer maize is subject to alterations in response to variations in soil conditions and cultivation practices, whereby tillage technique, sowing depth, planting density, and fertilizer conveyance all exert notable impacts on root growth (Guan et al., 2014; Wang et al., 2014; Cao et al., 2015). The growth and development of the root system were significantly influenced by planting density. As density increased, the growth space for the maize root system decreased (Chen et al., 2017). The findings of this study demonstrate that the cultivation modes of SH and HH

TABLE 6 Correlation analysis of yield with WUE, Pn, SPAD, LAI and root index.

Correlation index		Yield	WUE	Pn				LAI			SPAD				Root index			
				T1	T2	T3	T4	T1	T2	T3	T1	T2	T3	T4	L	D	T	S
Yield		1																
WUE		0.79**	1															
Pn	T1	0.19	0.33*	1														
	T2	0.09	0.11	0.91**	1													
	T3	0.60**	0.80**	0.43**	0.24	1												
	T4	0.81**	0.85**	0.11	0.3	0.68**	1											
LAI	T1	0.44**	0.66**	0.83**	0.74**	0.72**	0.27	1										
	T2	0.66**	0.77**	0.71**	0.56**	0.78**	0.49**	0.92**	1									
	T3	0.71**	0.81**	0.68**	0.53**	0.79**	0.54**	0.89**	0.97**	1								
SPAD	T1	0.76**	0.84**	0.49**	0.31	0.81**	0.69**	0.78**	0.94**	0.94**	1							
	T2	0.77**	0.81**	0.51**	0.33	0.83**	0.66**	0.77**	0.93**	0.93**	0.98**	1						
	T3	0.76**	0.83**	0.52**	0.35*	0.86**	0.68**	0.79**	0.93**	0.93**	0.98**	0.98**	1					
	T4	0.86**	0.84**	0.47**	0.31	0.81**	0.73**	0.73**	0.91**	0.91**	0.96**	0.97**	0.97**	1				
Root index	L	0.82**	0.89**	0.60**	0.40*	0.82**	0.68**	0.77**	0.88**	0.91**	0.89**	0.90**	0.90**	0.93**	1			
	D	0.72**	0.64**	0.52**	0.38*	0.59**	0.46**	0.68**	0.82**	0.83**	0.81**	0.82**	0.78**	0.81**	0.76**	1		
	T	0.84**	0.830**	0.61**	0.45**	0.76**	0.62**	0.81**	0.94**	0.95**	0.93**	0.93**	0.92**	0.95**	0.95**	0.87**	1	
	S	0.81**	0.675**	0.60**	0.49**	0.63**	0.50**	0.74**	0.88**	0.89**	0.85**	0.86**	0.84**	0.89**	0.85**	0.89**	0.960**	1

1: seedling stage, T2: jointing stage, T3: silking stage, T4: filling stage. L, root length; D, root diameter; T, number of root tips; S, root surface area.
*indicate that the different year is significantly different among materials (P<0.05), **indicate that the different site is significantly different among materials (P<0.01).

cultivation modes resulted in significantly higher root length, root surface area, root tip number, and root diameter compared to FP and CK cultivation modes. These results indicate that SH and HH cultivation modes effectively enhance the growth and development of maize root systems, leading to improved root system absorption performance. Therefore, given the prevailing circumstance of diminishing agricultural land, altering the cultivation mode emerges as the primary determinant for augmenting maize yield. Specifically, enhancing fertility, strategically planning planting density, and implementing mulching techniques are crucial in elevating maize yield in the northern region.

5 Conclusion

The results demonstrate that the super-high yield (SH) cultivation mode significantly outperformed the farmer mode (CK) in terms of yield, net photosynthetic rate, leaf area index, and SPAD values. This study showed that SH cultivation mode was a cultivation mode on the semiarid Loess Plateau. These findings suggest that enhancing the maize population through strategies such as increased planting density, appropriate fertilization, and mulching can effectively enhance maize yield and improve light and temperature utilization efficiency, ultimately leading to higher maize productivity and efficiency.

Data availability statement

The original contributions presented in the study are included in the article/supplementary materials, further inquiries can be directed to the corresponding author.

Author contributions

SW: Conceptualization, Investigation, Methodology, Software, Writing – original draft, Writing – review & editing. TF: Resources,

Visualization, Writing – review & editing. GZha: Project administration, Formal analysis, Writing – review & editing. MM: Formal analysis, Writing – review & editing. KL: Formal analysis, Project administration, Writing – review & editing. SL: Methodology, Project administration, Data curation, Writing – review & editing. WC: Funding acquisition, Project administration, Writing – review & editing. YD: Project administration, Writing – review & editing. LW: Conceptualization, Data curation, Writing – review & editing. JZ: Data curation, Investigation, Writing – review & editing. GZho: Methodology, Validation, Writing – review & editing. XL: Project administration, Validation, Writing – review & editing. SN: Methodology, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This work was supported by the National Natural Science Foundation of Corn Industry Technology System of the Ministry of Agriculture (GARS-02-66).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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OPEN ACCESS

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RECEIVED 01 August 2024

ACCEPTED 11 October 2024

PUBLISHED 04 November 2024

CITATION

Torabian S, Zakeri H and Farhangi-Abriz S
(2024) Optimizing faba bean cultivation:
assessing varietal performance in
spring and fall.
Front. Agron. 6:1474528.
doi: 10.3389/fagro.2024.1474528

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Optimizing faba bean cultivation: assessing varietal performance in spring and fall

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The need to identify specialty crops in Virginia has driven interest in faba beans (*Vicia faba* L.), which offer potential benefits for crop rotation systems. As a cool-season crop, faba beans can be planted in both fall and spring, providing flexibility in farming schedules. A field study was conducted at Randolph Farm, the Virginia State University Research and Extension Farm, using a completely randomized factorial block design. This study examines the performance of seven faba bean varieties—Ethiopia, NEB247, Aprovecho, EN3, EN47, Windsor and EN45—across three spring (late February, late March and mid April), and three fall (late September, early October and late October) planting dates. Our results demonstrate that both variety and planting date significantly influence the yield and yield components of faba beans. Among the varieties tested, Windsor and EN47 exhibited superior traits across multiple categories, making them preferable for achieving high yields. Conversely, varieties such as EN45, Aprovecho, and NEB247 showed poor performance. Fall planting dates generally resulted in superior growth, yield, and maturity characteristics, underscoring their importance for maximizing faba bean production. We observed that faba beans planted in the fall had 58% more branches, 100% more shoot dry matter, 34% higher 100-seed weight, double the grain yields, and 8% higher harvest index compared to those planted in the spring. To further enhance faba bean production, additional studies are suggested to clarify the physiological relationships between photosynthesis rates and the sink-source dynamics. Furthermore, investigating how planting dates impact the nutrient components of faba beans will provide deeper insights into optimizing their cultivation.

KEYWORDS

grain yield, harvest index, pod yield, Windsor, 100-seed weight

1 Introduction

Faba bean (*Vicia faba*), also known as broad bean or horse bean, is grown worldwide in cropping systems as a grain (pulse) and green-manure legume. It is the fourth most important pulse crop in the world and a popular vegetable in the Middle East and Europe, though uncommon in the U.S. In 2020, the world production of faba beans reached 5.67 million metric tons, a significant increase from 4.35 million metric tons in 1990. Major producers of faba beans include China, Ethiopia, France, Egypt, and Australia (Akibode and Maredia, 2012). Faba beans have been cultivated for thousands of years and are valued for their high protein content, nitrogen-fixing ability, and adaptability to various climatic conditions (Stoddard et al., 2010). As a legume, faba beans play a crucial role in sustainable agriculture by improving soil fertility and reducing the need for synthetic fertilizers (Crews and Peoples, 2005). In recent years, there has been a resurgence of interest in faba beans due to their potential to contribute to food security and environmental sustainability (Duc et al., 2015). Faba beans contain almost twice the protein content as cereal grains, with globulins (60%), albumins (20%), glutelins (15%), and prolamins (8%) (Rahate et al., 2021). Faba bean possesses high protein content from 20% to 41%; the wide variations are due to varietal differences and the source type, that is, flour, fraction, or isolate, as well as fertilization method, growth season, and planting site. In comparison with other beans such as lima, pinto, and red kidney beans, faba bean flour had the highest protein content of 29.76% (Gu et al., 2020).

Virginia, with its diverse climatic conditions and soil types, presents a unique opportunity to study the performance of different faba bean varieties under varying planting dates. The state's climate ranges from humid subtropical in the southeast to humid continental in the northwest, providing a broad spectrum of growing conditions (Cathey, 1990). The current state of crop rotation practices in Virginia's agricultural sector presents significant challenges for farmers. The predominant reliance on crops like rye, corn, hay, or grass has proven to be economically unviable for many growers. This limited diversification not only hampers farmers' profitability but also contributes to suboptimal soil health, making the agricultural systems more susceptible to diseases, pests, and weeds. The integration of alternative crops, such as faba beans, into the rotation systems could address these issues. However, the successful cultivation and marketing of faba beans depend on identifying the optimum planting dates and suitable varieties. Sowing date is a crucial determinant of crop yield, which is essential for increasing the productivity of various agronomic crops (Joshi et al., 2017; Refay, 2001; Wani et al., 2018). The recommendation for an optimal planting date depends on a combination of factors, including plant variety, temperature suitability, and water availability (Balalić et al., 2012). Environmental factors significantly influence plant growth and yield components, making the sowing date pivotal for sustainable grain yield and quality (Abbas et al., 2019). Adapting an optimum planting date is particularly important for new crops introduced to a region, ensuring favorable growing conditions.

The faba bean, a cool-season annual legume (Jensen et al., 2010), exemplifies this necessity. In California, it is typically planted

in February and March for vegetable use and from September to November for cover crops. The temperature range for growth is 5–35°C with an optimum temperature for photosynthesis of 25°C. Flowering is destroyed by frost, and few cultivars can tolerate temperatures <−10°C (Boote et al., 2002; Mínguez and Rubiales, 2021). Current faba bean cultivars are categorized in two main ways. First, they are classified as spring, Mediterranean, and winter types based on their vernalization requirements for flowering—none, mild, or strong, respectively. This classification allows for adaptation to various climates: spring types for cold and warm regions, Mediterranean types for areas without severe winters, and winter types for regions with cold winters that do not severely harm the crop. Second, cultivars are categorized by growth habit as indeterminate, semideterminate, and determinate, corresponding to long, short, and no vegetative growth after the last flower, respectively (Mínguez and Rubiales, 2021). Therefore, the choice between winter and spring faba beans heavily depends on variety, climate, soil type, and cropping system. Winter beans utilize autumn and winter moisture and mature early. Conversely, spring beans, vulnerable to summer drought, depend on early summer precipitation for high yields, making early sowing critical (Zhao et al., 2024). Planting date affects the phenological development of faba beans and their exposure to various biotic and abiotic stresses, such as temperature fluctuations, disease pressure, and pest infestations. Spring planting generally exposes crops to warmer temperatures and longer day lengths, enhancing vegetative growth and yield potential (Link et al., 1996). Conversely, fall planting can take advantage of cooler temperatures and reduced disease pressure but leaves crops more vulnerable to frost and shorter growing periods (Stoddard et al., 2010). Previous research underscores that genetic diversity within faba beans significantly influences their performance under different environmental conditions (Temesgen et al., 2015).

This study aims to investigate the performance of different faba bean varieties under varying spring and fall planting dates in Virginia, and it is the first to evaluate the combined effects of these factors on agronomic performance under local conditions. By systematically evaluating the growth characteristics, yield potential, and resilience of these varieties, the research seeks to identify optimal planting strategies that can enhance crop performance and sustainability.

2 Materials and methods

The experiment was conducted at Randolph Farm, the Virginia State University Research and Extension Farm in Chesterfield County, Virginia (37°13'43" N; 77°26'2" W) from 2023–2024. The study employed a completely randomized factorial block design with three replicates to evaluate the performance of seven faba bean varieties ('Ethiopia', 'NEB247', 'Aprovecho', 'EN3', 'EN47', 'Windsor' and 'EN45'). The study included three spring planting dates: February 24, 2023 and February 29, 2024 (late February), March 24, 2023 and March 21, 2024 (late March), and April 12, 2023 and April 12, 2024 (mid April). Additionally, three fall planting dates were used: September 22, 2023 (late September),

October 6, 2023 (early October), and October 22, 2023 (late October). Table 1 presents the plant introduction numbers for various faba bean varieties planted at the Research and Extension Randolph Farm.

Data on monthly mean air temperature (°C) and monthly precipitation (mm) were provided by the Weather Underground (<https://www.wunderground.com/history/monthly/us/va/petersburg>) located at Richmond International Airport Station (Figure 1).

The soil was tilled with a disk to ensure it was soft and even for planting. Baseline soil conditions were established by collecting soil samples from the field before planting, with the results presented in Table 2. To manage weed pressure, pre-planting herbicides such as Treflan (trifluralin) and S-metolachlor (Dual II Magnum) were applied at a rate of 1100 ml ha⁻¹ to control annual grasses and small-seeded broadleaf weeds. Fungicide Ridomil Gold® EC (Syngenta Crop Protection) at a rate of 1100 ml ha⁻¹ was applied to control soilborne oomycete diseases.

Each experimental plot measured 1.6 m × 2.4 m, with an additional 1 m buffer zone. Two rows were hand-planted in each plot at a depth of 5 cm. The space between rows was 38 cm, and the space between plants within each row was 15 cm, resulting in a plant population of approximately 11 plants m⁻². No seed inoculation or irrigation was performed during the experiment. Before planting, seeds were treated with Vibrance Maxx Seed Treatment (Syngenta US) at a rate of 0.1 ml per 100 g of seed to protect against damage from various soilborne, seed-borne, and seedling diseases. Hand weeding was carried out throughout the growing seasons. After germination and once the plants were adequately established, 40 kg nitrogen ha⁻¹ from urea, 30 kg P₂O₅ ha⁻¹ from triple superphosphate, and 40 kg potassium ha⁻¹ from K₂O were applied by hand throughout each plot. Urea was applied as a starter to promote early growth and nodulation, acknowledging faba bean's natural nitrogen-fixing ability. Throughout the growing season, data on germination, growth, performance, and days to harvest were recorded. Upon harvesting, yield and yield components were measured and documented. During the maturity stage, three plants were manually harvested from each plot between early March and early July 2024, and the average data for each plot was calculated. Samples were then bagged and dried in a Grieve forced-air oven at 65°C for 72 hours to obtain shoot and root dry weights and for further analysis. The shoot and root of each plant were weighed separately, and the number of branches was counted.

TABLE 1 Plant introduction of faba bean varieties planted at the Research and Extension Randolph Farm, Virginia State University.

Genotype	GRIN USA Plant Introduction
EN45	PI 655333
EN47	PI 568235
EN3	PI 254006
NEB247	PI 655333
Ethiopia	PI 371803
Windsor	PI 433531
Aprovecho	–

The number of pods per plant, pod weight per plant, number of seeds per pod, and number of seeds per plant were recorded. Yield per plant and yield per pod were obtained, and 100-seed weights were measured using a weighing scale. The harvest index was calculated using the equation:

$$\text{Harvest index} = \frac{\text{Grain yield (g)}}{\text{Total shoot dry weight(g)}} \times 100$$

According to our observations, the Aprovecho variety planted in mid-April and the NEB247 variety planted in mid-October died, resulting in no data for these varieties on those specific planting dates. Data from two years of spring plantings were pooled and analyzed together with data from a single fall planting date. A factorial randomized complete block design was employed, and a combined analysis of variance was conducted using SAS software (SAS Institute Inc, 2013). The least significant difference (LSD) at $P \leq 0.05$ was employed to compare the means in this study.

3 Results

3.1 Plant height

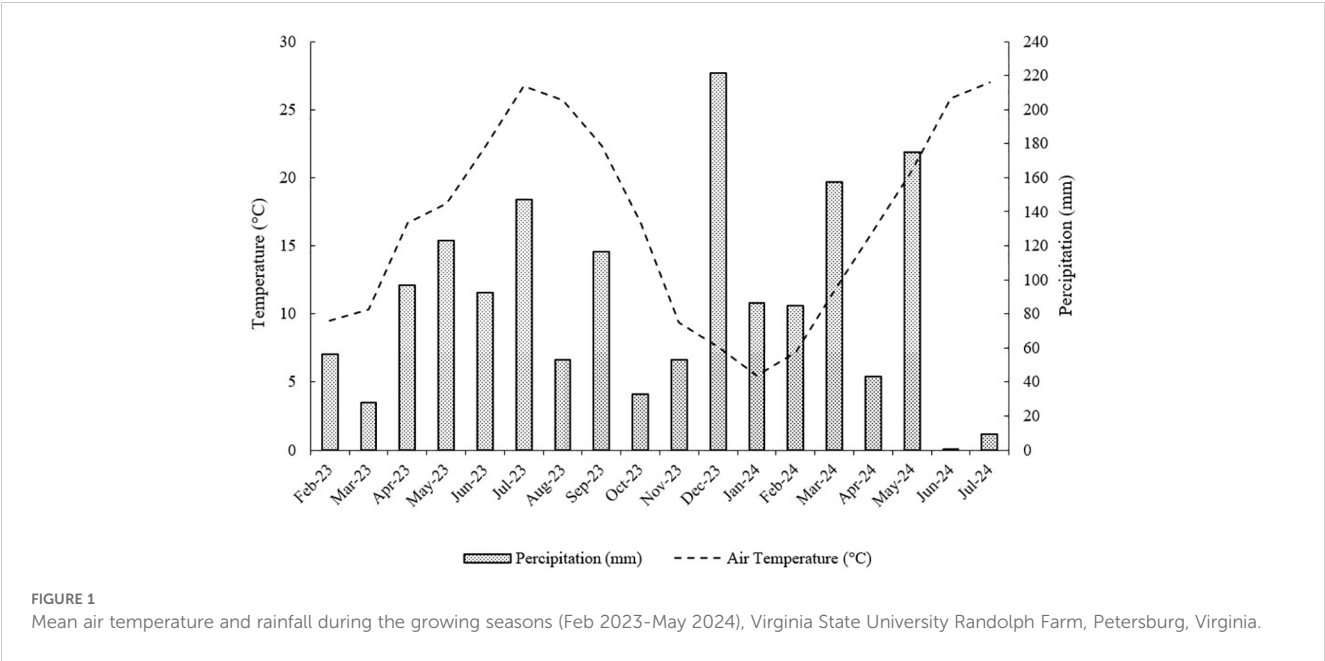
Analysis of variance indicated that both variety and planting date significantly ($P < 0.01$) affected the plant height of faba beans (Table 3). The comparison of mean values showed that Windsor (54.5 cm) and NEB247 (54.3 cm) had the highest plant heights, followed by Aprovecho (53 cm). Conversely, EN45 had the lowest plant height (32.5 cm) among the varieties. Across all varieties, faba beans planted in late September exhibited the highest plant heights. On average, fall planting dates resulted in greater growth and higher plant heights compared to spring planting dates. No interaction effects of variety and planting dates were observed (Table 3).

3.2 Shoot dry weight

The effects of variety and planting date on the shoot dry weight of faba bean samples are shown in Table 3. The maximum shoot dry matter was observed in Windsor (40 g), while EN45 had the lowest (7.96 g). According to the results presented in Table 3, the highest shoot dry matter was produced when faba beans were planted in late September. There was no significant difference in shoot dry matter between the spring planting dates (Table 3).

3.3 Day to maturity

As shown in Table 3, the maturity time of faba beans was significantly influenced by variety, planting date, and their interactions ($P < 0.01$). Regardless of planting date, NEB247 and Aprovecho had the longest maturity times, with 189 and 187 days, respectively. Conversely, EN3 and Windsor had the shortest maturity times, with 164 and 165 days, respectively. Faba beans planted in late September exhibited the longest maturity times (239 days) compared to other planting dates. However, mid-April



showed the shortest maturity time (89.3 days). On average, fall planting allowed for a longer growth period. Figure 2 illustrates the interactions between varieties and planting dates on maturity time. Across all varieties, the trend was consistent: late September planting resulted in the longest maturity time, while mid-April planting resulted in the shortest time to harvest.

3.4 Number of branches per plant

The number of branches in faba beans was significantly ($P < 0.01$) influenced by both variety and planting date (Table 3). Among the varieties, Aprovecho (3.83) and Windsor (3.54) had the highest number of branches. Across all varieties, faba beans planted in late September produced more branches compared to other planting dates. On average, fall planting resulted in a higher number of branches compared to spring planting dates (Table 3). There were no observed interaction effects between variety and planting dates.

3.5 Number of pods per plant

According to the ANOVA Table (3), the number of pods per plant was significantly affected by variety and planting dates ($P < 0.01$), and their interactions ($P < 0.05$). Between varieties, EN3 had the highest number of pods per plant (15.6); however, Aprovecho had the lowest (5.63). Across varieties, the highest number of pods per plant was recorded for plants planted late-Sep with 14.7.

Conversely, the lowest number of pods per plant was related to mid-April, with 4.76 (Table 3). The bar chart illustrates the number of pods per plant for seven faba bean varieties across six planting dates, which include three fall plantings and three spring plantings. According to Figure 3, EN3 shows the highest number of pods per plant for late-Sep planting dates. The number of pods per plant in all varieties except NEB247 was higher in the fall compared to spring planting dates (Figure 3).

3.6 Pod weight per plant

The ANOVA results (Table 3) indicated significant effects of variety and planting dates on pod weight per plant ($P < 0.01$), with no observed interaction effects. Across all planting dates, Windsor exhibited the highest pod weight at 25.4 g, while EN45 showed the lowest at 5.73 g (Table 3). Faba beans planted in late September exhibited the highest pod weight at 20.4 g, which did not significantly differ from those planted in early October (19 g) and late October (18.2 g). In contrast, the lowest pod weight was observed for faba beans planted in late March at 7.45 g, which was statistically similar to those planted in mid-April and late March (10.5 g) (Table 3).

3.7 Number of seeds per pod

The number of seeds per pod was significantly influenced by both variety and planting dates ($P < 0.01$), with no significant

TABLE 2 Soil chemical properties at Randolph farm (Pre-planting analysis).

CEC	pH	Acidity	Base saturation	Organic matter	N	P	K	Ca	Mg	Zn	Mn	Cu	Fe	B
meq/100g		%			mg kg ⁻¹					ppm				
2	6.4	2	98	1.5	12	56	56	314	25	0.4	4.9	0.3	25.1	0.1

TABLE 3 Analysis of variance (*P* values) on the effects of variety, planting date, and their two-way interactions on faba bean growth, yield and yield components.

	Plant height (cm)	Shoot dry weight (g)	Day to maturity	Number of branches per plant	Number of pods per plant	Pod weight per plant	Number of seeds per pod	Number of seeds per plant	100-seed weight (g)	Yield per plant (g)	Yield per pod (g)	Harvest index (%)
Rep	ns	ns	ns	ns	ns	ns	ns	ns	0.01	ns	0.01	ns
Variety (V)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	<0.01	<0.01	<0.01	<0.01
Planting date (P)	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	0.01	<0.01	0.02	0.02	ns	<0.01
V * P	ns	ns	<0.01	ns	0.03	ns	ns	ns	ns	ns	ns	0.04
Variety												
Ethiopia	47.9 ^{BC}	15.6 ^{CD}	179 ^C	2.94 ^{AB}	7.87 ^{BCD}	8.61 ^{CD}	1.97 ^B	15.1 ^{BC}	39.2 ^D	6.27 ^{CD}	0.79 ^{CD}	38.2 ^B
NEB247	54.3 ^A	18.8 ^{BC}	189 ^A	3.24 ^{AB}	9.07 ^{BC}	9.08 ^{CD}	1.91 ^B	17.4 ^{BC}	36.6 ^D	6.38 ^{BCD}	0.74 ^{CD}	32.9 ^{BC}
Aprovecho	53.0 ^{AB}	26.4 ^B	187 ^A	3.83 ^A	5.63 ^D	11.4 ^{BCD}	2.01 ^B	10.2 ^C	56.3 ^C	8.02 ^{BCD}	1.73 ^B	30.5 ^C
EN3	46.8 ^C	19.5 ^{BC}	164 ^E	3.15 ^{AB}	15.6 ^A	12.7 ^{BC}	1.90 ^B	29.4 ^A	32.5 ^D	9.74 ^{BC}	0.63 ^D	51.1 ^A
EN47	41.4 ^D	21.0 ^{BC}	184 ^B	2.58 ^B	9.95 ^B	15.2 ^B	1.66 ^B	15.0 ^{BC}	73.6 ^B	11.0 ^B	1.30 ^{BC}	52.2 ^A
EN45	32.5 ^E	7.96 ^D	173 ^D	2.43 ^B	8.42 ^{BCD}	5.73 ^D	1.79 ^B	15.2 ^{BC}	27.1 ^D	4.37 ^D	0.54 ^D	48.9 ^A
Windsor	54.5 ^A	40.0 ^A	165 ^E	3.54 ^A	6.64 ^{CD}	25.4 ^A	3.03 ^A	20.3 ^B	93.5 ^A	18.7 ^A	2.89 ^A	46.5 ^A
Planting date												
Late February	43.5 ^C	15.0 ^C	109 ^D	1.74 ^C	8.21 ^B	10.5 ^B	2.05 ^C	15.5 ^{BC}	52.0 ^B	7.88 ^B	1.12	50.8 ^A
Late March	44.7 ^{BC}	13.8 ^C	95.1 ^E	2.27 ^C	6.49 ^C	7.45 ^B	1.93 ^C	12.5 ^C	44.4 ^B	5.33 ^B	1.04	37.5 ^B
Mid-April	42.6 ^C	18.0 ^C	89.3 ^F	3.21 ^B	4.76 ^C	10.5 ^B	2.24 ^{BC}	11.4 ^C	54.8 ^B	7.65 ^B	1.41	36.0 ^B
Late September	55.0 ^A	39.6 ^A	239 ^A	6.03 ^A	14.7 ^A	20.4 ^A	1.918 ^C	26.3 ^A	52.9 ^B	14.2 ^A	1.28	34.5 ^B
Early October	48.9 ^B	28.2 ^B	229 ^B	3.21 ^B	8.50 ^B	19.0 ^A	2.45 ^{AB}	20.5 ^{AB}	73.7 ^A	14.6 ^A	2.00	49.6 ^A
Late October	47.2 ^{BC}	27.1 ^B	218 ^C	2.24 ^C	6.68 ^C	18.2 ^A	2.64 ^A	17.4 ^{BC}	76.0 ^A	13.4 ^A	2.16	50.6 ^A

Different letters within columns in each parameter indicate significant differences by the least significant difference (LSD) test at *P* < 0.05. ns, non-significant.

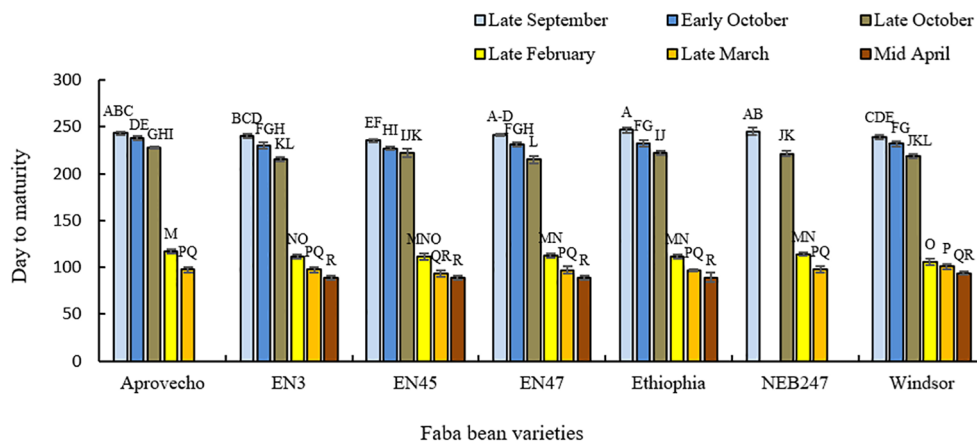


FIGURE 2

The interaction effects of varieties and three spring planting dates (late February, late March, and mid-April) and three fall planting dates (late September, early October, and late October) on the day to maturity for faba beans. Different letters indicate significant differences by the least significant difference (LSD) test at $P < 0.05$. Bars on the columns are means $\pm$ standard error.

interactions ($P > 0.05$) (Table 3). Windsor had the highest number of seeds per pod (3.03), with no significant difference between the other varieties. Across all varieties, the highest number of seeds per pod was recorded for faba beans planted in late October (2.64), which did not significantly differ from those planted in early October (2.45) (Table 3).

3.8 Number of seeds per plant

The number of seeds per plant was significantly influenced by both variety and planting dates ($P < 0.01$), with no significant interactions (Table 3). Among the varieties, EN3 recorded the highest number of seeds per plant (29.4), followed by Windsor (20.3); whereas Aprovecho exhibited the lowest (10.2). Faba beans planted in late September had the highest seed number per plant

(26.3), which was not significantly different from those planted in early October (20.5). Conversely, the lowest seed number per plant was observed in spring plantings, particularly in mid-April (11.4) (Table 3).

3.9 100-Seed weight

The 100-seed weight was significantly influenced by both variety ($P < 0.01$) and planting date ($P < 0.05$), with no significant interaction effects (Table 3). Among the varieties, Windsor had the highest 100-seed weight (93.5 g), followed by EN47 (73.6 g), while EN45 had the lowest (27.1 g). Across all varieties, the highest 100-seed weight was recorded for faba beans planted in late October (76 g), which was not significantly different from those planted in early October (73.7 g) (Table 3).

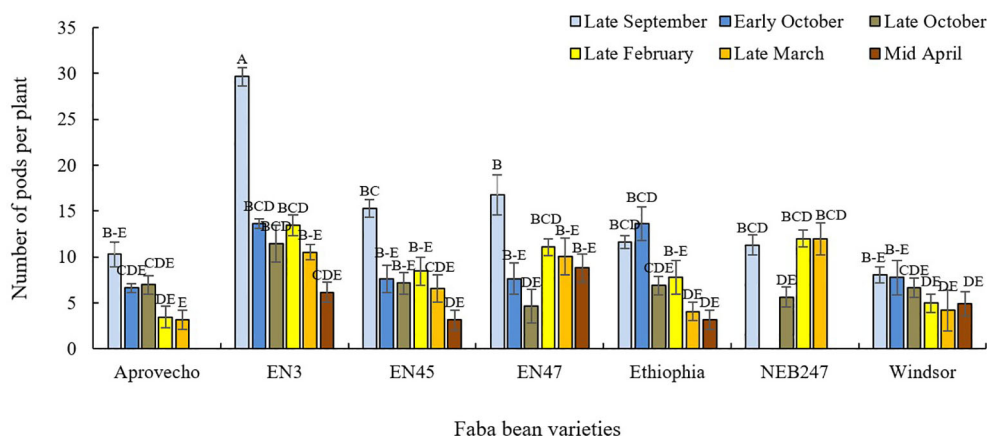


FIGURE 3

The interaction effects of varieties and three spring planting dates (late February, late March, and mid-April) and three fall planting dates (late September, early October, and late October) on the number of pods per plant for faba beans. Different letters indicate significant differences by the least significant difference (LSD) test at $P < 0.05$. Bars on the columns are means $\pm$ standard error.

3.10 Yield per plant

According to the ANOVA (Table 3), the yield of faba beans was significantly affected by variety ($P < 0.01$) and planting date ($P < 0.05$), but their interactions were not significant. Regardless of planting date, Windsor had the highest grain yield (18.7 g) compared to other varieties, while EN45 had the lowest yield (4.37 g). Across all varieties, the three fall planting dates resulted in the highest faba bean yields, which were approximately 100% higher on average than those planted in spring. There was no statistical difference between spring planting dates in terms of faba bean yield.

3.11 Yield per pod

Table 3 illustrates that variety has a significant effect ($P < 0.01$) on faba bean yield per pod; however, there was no significant effect of planting date or their interactions. The results showed that Windsor (2.89 g) had the highest yield per pod among the tested faba bean varieties, while EN45 (0.54 g) had the lowest yield per pod. The data indicated that faba beans planted in the fall had a slightly higher yield per pod compared to those planted in the spring, but this difference was not statistically significant (Table 3).

3.12 Harvest index

According to the ANOVA Table (3), the harvest index was significantly affected by both variety and planting dates ($P < 0.01$), as well as their interactions ($P < 0.05$). EN47 had the highest harvest index (52.2%), which was not significantly different from that of EN3 (51.1%), EN45 (48.9%), and Windsor (46.5%). The lowest harvest index was recorded for the variety Aprovecho, with a value

of 30.5%. Across all varieties, the harvest index was highest for faba beans planted in late February (50.8%), early October (49.6%), and late October (50.6%) (Table 3). The bar chart illustrates the interactions among seven faba bean varieties across six planting dates with respect to the harvest index (Figure 4). EN47 planted in late October (61.1%) and EN45 planted in late February (60.9%) achieved the highest harvest index. Conversely, NEB247 and Aprovecho planted in late September had the lowest harvest index, which was around 10% (Figure 4).

4 Discussion

This scatter plot illustrates the relationship between yield per plant (g) and various yield components of faba beans (Figure 5). There is a positive correlation between yield and components such as pod weight per plant ($r = 0.98$, $P < 0.01$), number of pods per plant ($r = 0.40$, $P < 0.01$), number of seeds per pod ($r = 0.55$, $P < 0.01$), number of seeds per plant ($r = 0.65$, $P < 0.01$), harvest index ($r = 0.49$, $P < 0.01$), 100-seed weight ($r = 0.56$, $P < 0.01$), and yield per pod ($r = 0.66$, $P < 0.01$). The red dotted trend line suggests a linear relationship between yield and components like pod weight, number of pods per plant, number of seeds per pod, and yield per pod, supporting findings by Alan and Geren (2007) and Aziz et al. (2013) that these components often exhibit linear relationships with yield. However, the relationship between yield and the number of seeds per plant, harvest index, and 100-seed weight were non-linear. Among the yield components, pod weight per plant shows the strongest correlation with yield. Studies have shown that pod weight per plant is a significant determinant of overall yield in faba beans, indicating a strong positive correlation (Ulukan et al., 2003; Sindhu et al., 1985; Berhe et al., 1998).

The study provides detailed insights into how variety and planting date affects various agronomic traits of faba beans, such as plant height, shoot dry weight, days to maturity, number of branches

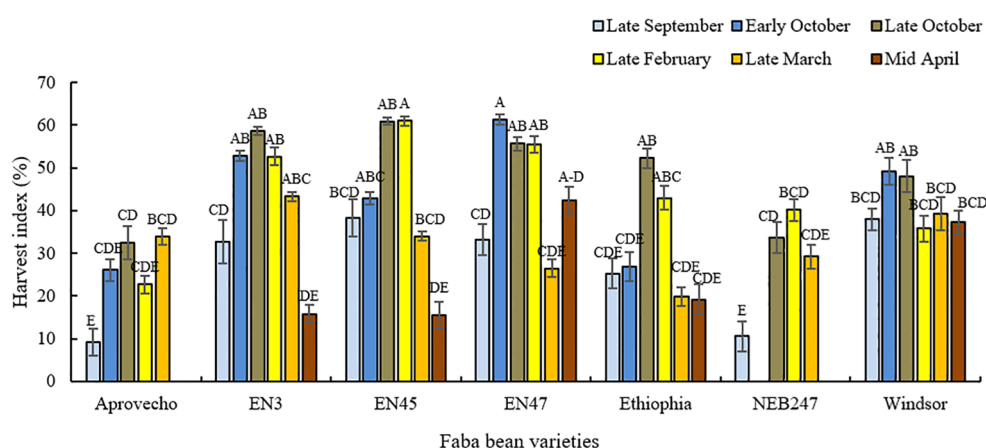


FIGURE 4

The interaction effects of varieties and three spring planting dates (late February, late March, and mid-April) and three fall planting dates (late September, early October, and late October) on the number of pods per plant for faba beans. Different letters indicate significant differences by the least significant difference (LSD) test at $P < 0.05$. Bars on the columns are means $\pm$ standard error.

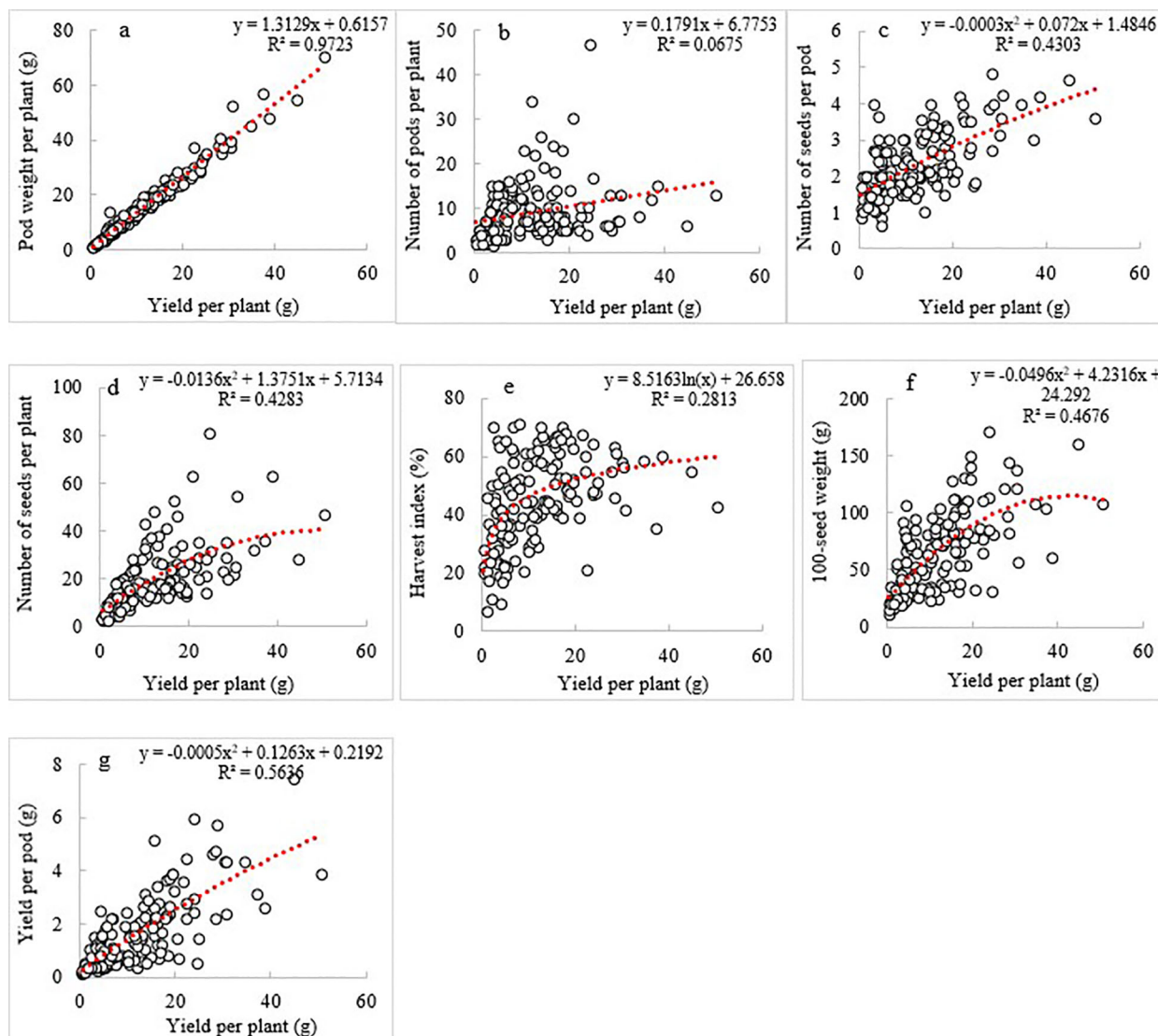


FIGURE 5

The scatter plots for faba bean yield in relationship with pod weight per plant (A), number of pods per plant (B), number of seeds per pod (C), number of seeds per plant (D), harvest index (E), 100-seed weight (F), and yield per pod (G).

per plant, number of pods per plant, pod weight per plant, number of seeds per pod, number of seeds per plant, 100-seed weight, yield per plant, yield per pod, and harvest index. Among the varieties, Windsor demonstrated superior performance in several key areas. It achieved the highest plant height (54.5 cm), shoot dry matter (40 g), number of branches (3.54), pod weight (25.4 g), number of seeds per pod (3.03), 100-seed weight (93.5 g), grain yield (18.7 g), and yield per pod (2.89 g). Additionally, Windsor had the shortest maturity time compared to other varieties. These characteristics make Windsor an excellent choice for maximizing yield and efficiency. Following Windsor, EN47 showed commendable performance with the highest harvest index (52.2%). Given the strong correlation between pod weight, 100-seed weight, and grain yield, both Windsor and EN47 emerged as superior varieties compared to others. This correlation highlights the

importance of these traits in determining overall yield performance (Duc, 1997). In contrast, EN45 exhibited the lowest values in several critical areas, including plant height, shoot dry weight, pod weight, 100-seed weight, and grain yield. These deficiencies suggest that EN45 is not well-suited for the conditions of this study. Similarly, the varieties Aprovecho and NEB247 showed specific sensitivities to planting dates. Aprovecho, when planted in mid-April, and NEB247, when planted in early October, both failed to thrive, indicating a sensitivity to hot and cold weather, respectively. This sensitivity makes these varieties less suitable for regions with extreme temperature variations. Aprovecho had the lowest number of pods per plant and the lowest harvest index (30.5%), and it also had the longest maturity time compared to other varieties. These factors further support the conclusion that Aprovecho is not an ideal variety

for the region under the conditions tested. The results indicated that the choice of variety significantly impacts the agronomic performance of faba beans. These findings are consistent with previous research, emphasizing the critical role of variety selection in optimizing crop performance (Jensen et al., 2010; Siddiqui et al., 2015; Afzal et al., 2022).

Across all varieties, the planting date significantly influenced the yield and yield components of faba beans (Table 3). Faba beans planted in late September exhibited the tallest plants, longest maturity times, more branches, highest shoot dry matter, most pods per plant, heaviest pod weight, and greatest number of seeds per plant compared to other planting dates (Table 3). Previous studies support our findings, indicating that optimal planting times can significantly influence vegetative growth and plant height (Wakweya et al., 2016; Refay, 2001; Turk and Tawaha, 2002). The extended growing period afforded by fall planting dates likely contributes to the longer maturity times observed (Ellis et al., 1992). Our results demonstrated that the shoot dry matter of faba beans planted in the fall was nearly 100% higher than those planted in the spring. This finding is consistent with Thalji and Shalaldeh (2006), who reported a significant yield advantage (157%) and increased shoot and root growth with early planting (end of November). We observed that faba beans planted in fall had 58% more branches compared to those planted in spring. As shown in Table 3, pod development for faba beans was higher for those planted in fall compared to spring, which aligns with previous studies indicating that fall planting dates result in greater pod development and weight (Jensen et al., 2010). This pattern suggests that fall planting dates provide favorable conditions for pod formation, supported by research from Loss and Siddique (1997). El-Metwally et al. (2013) found that sowing on October 25th produced the highest growth characteristics and pigment content (total chlorophyll), while the greatest yield and its components were achieved with the November 25th sowing date. The 100-seed weight of faba beans planted in the fall was approximately 34% higher than those planted in spring. Previous research has shown that environmental conditions during fall planting favor the development of larger seeds (Duc, 1997). The data showed that faba beans planted in the fall had a slightly higher yield per pod than those planted in the spring, although this difference was not statistically significant (Table 3). The three fall planting dates produced the highest faba bean yields and had a higher harvest index, averaging about 100% and 8% more than those planted in spring, respectively. This trend suggests that fall planting dates enhance seed production, consistent with findings by Khan et al. (2010). In the current study, some varieties (Ethiopia, NEB247, and Aprovecho) planted in late September entered the reproductive phase before winter. Being indeterminate, they continued to bloom even after losing their flowers in December and January. It is necessary to use indeterminate varieties for fall planting because if the weather conditions are favorable and encourage blooming, the plants are unlikely to retain their flowers

through the winter. Other varieties planted in the fall in this study remained in the growth stage and did not enter the reproductive stage before spring. The biggest challenges for spring planting include cold weather at the beginning of the season and rain, which prevent the soil from being ready for planting. Additionally, hot weather during the flowering stage of faba beans can hinder grain production. As the weather warms, disease problems such as chocolate spot and rust will spread more rapidly, favoring warmer temperatures of 15–25°C and above 20°C, respectively (Stoddard et al., 2010). Therefore, for spring planting, faba beans should be planted as soon as possible to avoid hot weather during the flowering stage.

5 Conclusion

The study demonstrates that both variety and planting date play critical roles in determining the agronomic performance of faba beans. Varieties like Windsor and EN47, which exhibit superior traits across multiple categories, are preferable for achieving high yields. Conversely, varieties such as EN45, Aprovecho, and NEB247, which show poor performance or sensitivity to adverse conditions, are less suitable. Fall planting dates generally result in superior growth, yield, and maturity characteristics, highlighting their importance for maximizing faba bean production. To maximize the agronomic performance and yield of faba beans, careful consideration must be given to both variety selection and planting date. However, given that this study was conducted in a single region and soil type, future research should extend these investigations to diverse environmental conditions to validate and generalize the findings. Additionally, further studies are needed to clarify the physiological relationship between photosynthesis rates and the sink-source relationship and to explore how planting dates impact the nutrient components of faba beans, such as amino acids, fat, and carbohydrates.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

ST: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. HZ: Writing – review & editing. SF: Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. We thank USDA-NIFA (7004952) for funding through the Evans-Allen Program.

Acknowledgments

We appreciate the technical support from the farm crew: David Jonson, Amanda Seow, and Diego M. Mayorga Valladares in field management. The authors acknowledge the use of ChatGPT (version: GPT-4, source: OpenAI) for text revision and editing assistance.

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OPEN ACCESS

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RECEIVED 26 September 2024

ACCEPTED 16 December 2024

PUBLISHED 22 January 2025

CITATION

Lopez-Nuñez R, Prieto-Rubio J, Bautista I,
Lidón-Cerezuela AL, Valverde-Urrea M,
Lopez-Moya F and Lopez-Llorca LV (2025)
Chitosan reduces naturally occurring plant
pathogenic fungi and increases
nematophagous fungus *Purpureocillium* in
soil under field conditions.
Front. Agron. 6:1502402.
doi: 10.3389/fagro.2024.1502402

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Chitosan reduces naturally occurring plant pathogenic fungi and increases nematophagous fungus *Purpureocillium* in soil under field conditions

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Chitosan effects on soil properties were analysed both under laboratory conditions by incubation with constant humidity and temperature and under field conditions in two persimmon field plots with conventional and ecological management. Chitosan was applied in solution or as coacervates. Application of chitosan reduced soil pH, conductivity (CE), and cation exchange capacity (CEC) in pots when applied at field capacity. Chitosan did not affect field soil respiration, which is greatly dependent of soil moisture and temperature. Metabarcoding showed that chitosan significantly modifies the fungal genera composition of ecologically managed field soil. On the contrary, chitosan caused no significant differences in bacterial taxa composition of soil under field conditions. Chitosan coacervates increased naturally occurring nematophagous fungus *Purpureocillium* (ca. 50-fold) in soil with respect to chitosan solution-treated soil and untreated controls. In addition, chitosan reduced the inoculum of plant pathogenic fungi *Alternaria* and *Fusarium* (20% and 50%, respectively) in field soil. Soil microbial network analysis for ITS2+V1–V2 regions revealed that the nematophagous fungus *Pochonia* promoted network clustering into modules. Furthermore, network analysis for ITS2+V3–V4 regions showed that the nematode trapping-fungus *Orbilia* and bacteria belonging to *Acidimicrobiales* and *Cytophagales* significantly contributed to network clustering in field soil. Our results show that chitosan coacervates increased soil nematophagous microbiota and that both nematode egg parasites and trapping fungi help to structure soil microbiota.

KEYWORDS

chitosan, metabarcoding, nematophagous fungi, plant pathogenic fungi, co-occurrence networks, coacervates

1 Introduction

The use of chemical pesticides, imposed by demographic changes, is the most common strategy to improve agricultural productivity. However, there is a trend towards the use of ecological additives, such as chitosan, with low environmental impact, instead of chemical synthesis agrochemicals such as nematicides (Bautista-Baños et al., 2005; Lopez-Nuñez et al., 2022). Chitosan is also a source of nitrogen for stimulating plant growth (Pichyangkura and Chadchawan, 2015). The behaviour of chitosan in soil is related to its cationic nature. This allows electrical interactions with the negatively charged surfaces of clay minerals, modifying its behaviour in soil (Hataf et al., 2018).

Chitosan can modify some soil properties (Reddy et al., 2018). This biopolymer can act as a cohesive agent for clay particles (Hataf et al., 2018). Arid soils are often low on natural polysaccharides, which stabilise soil structure (Orts et al., 2000). Chitosan can bind metal ions and limit their leachability, even in the presence of K^+ , Cl^- , and NO_3^- , the dominant ions in soil (Kamari et al., 2011). Furthermore, it can reduce the bioavailability of nickel (Turan, 2019; Heidari et al., 2020) and immobilises chromium when combined with other adsorbents (Najafi et al., 2021). Chitosan is a source of nitrogen, promoting plant growth (Pichyangkura and Chadchawan, 2015). Chitosan is also an elicitor of plant defences that can trigger physiological and structural responses in the plant, inducing jasmonic acid (JA) and salicylic acid (SA) production (Lopez-Moya et al., 2019; Suarez-Fernandez et al., 2020). Chitosan is active against plant pathogenic nematodes (Khalil and Badawy, 2012), has antiviral and antifungal activity, and induces tolerance to abiotic and biotic stresses in several horticultural crops (Iriti and Varoni, 2015; Malerba and Cerana, 2016).

Chitosan sensitivity of filamentous fungi and yeasts increases with carbon and nitrogen limitation (Lopez-Moya et al., 2015). Chitosan permeabilises the membrane of the fungus *Neurospora crassa*, in an energy-dependent manner. Conidia are most sensitive to chitosan membrane permeabilization followed by germlings and vegetative hyphae. Therefore, chitosan causes conidial lysis and death within minutes (Palma-Guerrero et al., 2009). Membrane fluidity is a key factor in fungal sensitivity to chitosan (Palma-Guerrero et al., 2010a; Zavala-González et al., 2016). Chitosan-sensitive fungi such as important plant pathogens (e.g., *Fusarium* spp. and *Alternaria* spp.) have a high content of polyunsaturated fatty acids (Ren et al., 2021; Chen et al., 2014). Plant diseases caused by species of the genus *Fusarium* consist of vascular wilts and consequent rotting of roots, stems, and the rest of the plant (Torres, 2000). Blight disease is one of the most dominant diseases causing an average yield loss of 32–57% caused by the *Alternaria* genus (Mamgain et al., 2013). In contrast, chitosan-resistant fungi such as nematophagous (e.g., *Pochonia*

chlamydosporia) or entomopathogens (e.g., *Beauveria bassiana*) have a lower presence of polyunsaturated fatty acids in membrane lipids. These fungi express, upon exposure to chitosan, extracellular hydrolytic enzymes (chitosanases, chitinases, and proteases) involved in nematode egg penetration. Furthermore, chitosan increases conidiation in nematophagous and entomopathogenic fungi (Palma-Guerrero et al., 2010b, 2010c).

Current work on chitosan biological activity of chitosan has focussed mostly on axenic systems. No data are available on the effect of chitosan on soil microbiota under natural conditions. Therefore, in this work, we studied the effect of chitosan on the abundance of ecological agriculture soil microbiota using metabarcoding and evaluated fungal and bacterial co-occurrence networks. The effects of chitosan solutions or coacervates on soil physicochemical properties were also studied both in the laboratory and in the field.

2 Materials and methods

2.1 Chitosan solutions and coacervates

Chitosan powder (Marine Bioproducts GmbH, Germany) was dissolved in 0.25 M HCl to obtain an initial concentration of 10 mg/mL, and pH was adjusted to 5.6. The resulting solution was then dialysed against distilled water for 2 days and autoclaved. Chitosan solutions were stored at 4°C until used for a maximum of 30 days. Control solutions were prepared likewise but without adding chitosan.

Chitosan was dissolved in sodium acetate buffer (pH 5) to obtain a 3% solution. Chitosan coacervates (T8C) were formed by dropping a 3% chitosan solution into 10% sodium hydroxide using a plastic syringe (Terumo Europe NV), with a 0.2-mm-diameter outlet. T8C were left for 5 min in the sodium hydroxide solution. T8C were then washed in sterile distilled water to reach pH 8. T8C were dried onto sterile filter paper in a laminar flow hood (Telstar BV-100) for 24 h. T8C were then stored at room temperature in sterile containers.

2.2 Application of chitosan to agricultural field soil

Persimmon fields in Pedralba (Valencia, E, Spain), conventionally (39° 35' 55.25'' N, 0° 43' 47.31' W) and ecologically (39° 35' 52.47'' N, 0° 43' 41.47' W) farmed, were selected for experiments (Table 1). Soil properties were determined; soil was air-dried soil and sieved through a 2-mm sieve. Soil pH was measured in a 1:2.5 (w/v) aqueous solution using a pH meter (2001, Crison, Barcelona, Spain). Electrical

TABLE 1 Physicochemical characteristics of the soils used in this study.

Type soil	Texture	BD (g/cm ³)	pH (H ₂ O)	EC 1:5 (dS/m)	CaCO ₃ (%)	OM (%)
A Cq E	Sandy loam	1.161	7.76 ± 0.03	0.40 ± 0.03	43.18 ± 0.12	13.05 ± 1.29
A Cq C	Loam	1.303	8.16 ± 0.08	0.20 ± 0.06	37.04 ± 1.82	3.42 ± 0.05

BD, bulk density; EC 1:5, electrical conductivity extracts 1:5; OM, organic matter; A CQ E, Pedralba persimmon ecological; A CQ C, Pedralba Persimmon Conventional (Lull et al., 2021).

conductivity was determined in a 1:5 (w/v) aqueous solution using a conductivity meter (model, Crison). The carbonate content was determined using a Bernard calcimeter. Soil organic matter (OM) was determined by wet oxidation using the Walkley–Black titration method (Walkley and Black, 1934). Soil texture was determined by the Bouyoucos method (Bouyoucos, 1927). Surface soil (0 cm–10 cm) from both plots was taken for the incubation experiment with pots in growth chambers. Also, these plots were used for an experiment of chitosan application in the field where two treatments were selected: coacervates, only one application at the beginning of the experiment and soluble chitosan applied monthly along 9 months with the dose divided between the number of applications.

2.2.1 Field experiments

Three 1 × 1 m plots were marked in each field (Figure 1). Each plot was subdivided into six 33 × 50 cm subplots. Three subplots per plot were randomly selected for treatments. These included Control (C) (no Chitosan), 1 mg/mL Chitosan solution (T8L), and Chitosan coacervates (T8C). Selected T8C subplots were treated with chitosan coacervates (9 g/subplot) at the start of the experiment. C and T8L subplots were irrigated monthly (1 L/subplot) for 9 months with either distilled water (C and T8C) or 1 mg/mL chitosan (T8L). Field soil moisture, temperature, and electrical conductivity were measured monthly (for nine months) using a WET-2 sensor (HH2 Moisture Meter, Delta-T Devices, Burwell, UK). Respiration rate and CO₂ concentration were also measured monthly (for 9 months) using an EGM-4 environmental gas monitor device (PP System Company, Amesbury, MA, USA). At the end of the experiment, four core samples were collected from each treated subplot with a cylindrical auger (5.35 cm in diameter and 12.77 cm in length). Soil cores were placed in 15 × 20 cm sterile airtight bags. Soil subsamples (10 g) were sieved through a 2-mm mesh and then air dried to measure cation exchange capacity, pH, soil moisture (see below), and mineral nitrogen. Soil mineral nitrogen (nitrate and ammonium) was extracted in 2 M KCl and analysed colorimetrically by flow injection (FIAstar 5000, Foss

Tecator, Höganäs, Sweden) (Rhoades, 1982). Cation exchange capacity was determined by the sodium acetate sodium chloride method (Rhoades, 1982).

2.2.2 Laboratory trials

Polystyrene cups (200 mL) with a hole in the base covered with glass wool were filled with soil collected from each of the Pedralba plots. Cups were incubated in a growth chamber (SANYO, MLR-351H) at 24°C and 60% relative humidity under a 16-h light/8-h dark photoperiod. Cups with soil were irrigated periodically (2–3 days) to maintain soil moisture to field capacity according to the texture of each soil (see below). There were 10 replicate pots set per soil (conventional and ecological management) and treatment: Control (C) (no Chitosan), at 1 mg/mL Chitosan solution (T8L), and Chitosan coacervates (T8C) 1 g/plot.

For the determination of the moisture of each soil at field capacity, we placed 12.5 cm of soil in a 15.5-cm-long and 3.5-cm-wide percolation tube. Water was then added to wet the first 5 cm of soil. The top of the tube was capped with Parafilm® and aluminium foil, leaving the tap open for 48 h–72 h. We then discarded the first centimetre of soil, took a sample of moist soil, and weighed it. We dried the soil at 105°C to constant weight. We calculated soil field capacity with the formula described in Llorca-Llorca (1991):

Soil Moisture at Field capacity = (Moist Soil Weight – Dry Soil Weight)/Dry Soil Weight

After 30 days, the soil from three pots per soil type and treatment was pooled and homogenised per triplicate (nine pots sampled). Then, soil humidity, pH, electrical conductivity, and cation exchange capacity were analysed. This experiment was carried out in duplicate.

2.3 Physicochemical analysis of soils

Soil samples for both regimes (ecological and conventional) were taken from each subplot and treatment for physicochemical

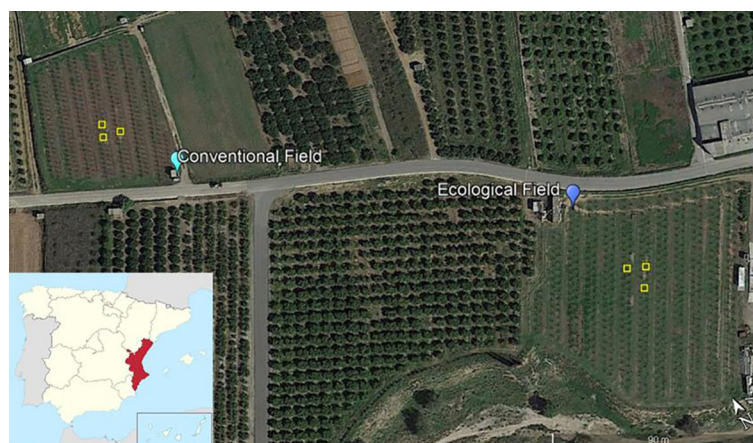


FIGURE 1

Persimmon experimental fields. Fields were in Pedralba, Valencian Community (East, Spain). Conventional Field received mineral fertilisation and usual agronomic practices. Ecological Field received organic fertilisation only, and no agrochemicals were applied. Experimental plots (1 × 1 m) where treatments were applied, and soil samples collected are marked by yellow boxes. Google Earth (2024). <https://www.google.com/earth/>.

determinations at the end of the experiment. Soil moisture, conductivity/salinity, pH, texture, and cation exchange capacity were determined for all soil samples collected (Llorca-Llorca, 1991). Three measurements were taken per each physicochemical parameter for treatment and soil type.

2.4 Soil metabarcoding

On the same day of collection (only for ecological soil), DNA was extracted from fresh soil (250 mg per soil sample), using DNeasy PowerSoil Pro Kit (Qiagen, Germantown, MD, USA). DNA samples were sent to Macrogen Inc. (Seoul, Korea), where they were amplified and using specific fungi (ITS2) and bacteria (V1–V2, V3–V4) primers (Table 2) and sequenced by the Illumina MiSeq platform using the v3 reagent kit. DNA reads obtained were analysed using the OmicsBox 3.0 package to identify the microorganisms present in soil samples. Metabarcoding data are available in the NCBI BioProject accession number PRJNA1164777 (<https://www.ncbi.nlm.nih.gov/sra/PRJNA1164777>).

2.5 Soil microbe co-occurrence networks

Fungal and bacterial communities characterised from ITS2, V1–V2, and V3–V4 amplicon sequencing were analysed through co-occurrence networks by using the SParse Inverse Covariance Estimation for Ecological Association Inference (SPIEC.EASI) pipeline in R package (Kurtz et al., 2015). This network-based approach allowed to frame both fungal and bacterial communities into a similar co-occurrence network (Wagg et al., 2019). Before network inferring, OTUs that occurred >1% and more than five samples were maintained in the datasets and rescaled to the proportion of the minimum sequencing depth (32,672 reads for fungi in the ITS2 dataset, 38,709 for bacteria in the V1–V2 and 38,011 for bacteria in the V3–V4). The inference was carried out by combining the amplicon pair dataset, ITS2+V1–V2 and ITS2+V3–V4. We fitted the spiec.easi function with Meinshausen–Bühlmann’s neighbourhood selection method, and the lambda minimum ratio at 0.01. From the spiec.easi object, we extracted the OTU adjacency matrix with the symBeta function to infer the network graphs and network properties of OTUS from the Gephi software (Bastian et al., 2009). In particular, we determined the degree centrality, which counts the number of links per OTU and its metric weighted by the occurrence frequency per linked OTU pairs (Gouveia et al., 2021); the modularity class for each OTU embedded

in the network, i.e., the module which an OTU belong to; and the clustering coefficient, which measures the extent of an OTU to cluster with others into a module (Latapy, 2008).

2.6 Statistical analysis

Results from pot tests were analysed with a three-way ANOVA to determine statistical differences for each variable tested (pH, electrical conductivity, cation exchange capacity), with the factors soil, treatment (fixed and orthogonal), and experiment (random and orthogonal) at the end of the experiment (30 days).

For the field test variables (pH, conductivity, cation exchange capacity, and mineral nitrogen), a two-way ANOVA of soil and treatment (fixed and orthogonal) was performed for the last data collection time of the field experiment (9 months).

Then, a three-way ANOVA was performed to analyse the differences of each variable (respiration rate, electrical conductivity, soil moisture, and soil temperature), with the factors soil, treatment, and time (fixed and orthogonal). The ANOVA requirements were tested with the DHARMA package (Hartig, 2022).

For the ecological soil metabarcoding analysis, the OmicsBox 3.0 program was used to obtain relative abundances of phylum, order, genus, and species for the ITS2, V1–V2, and V3–V4 primers, with the Kraken 2.1.2 function (Wood et al., 2019; Wood and Salzberg, 2014). Abundances above 1% (relative abundance) were taken for statistical analyses. The mean relative abundance and standard error were calculated with Excel.

To study the differences of phylum, genus, order, and species present in the ecological soil according to treatment, a multivariate generalised linear model (GLM) with a Gaussian distribution of the error (“manyglm” function in the “mvabund” package) was performed. A univariate GLM with a Gaussian family error distribution was then performed for each variable to analyse the differences between abundances in genera and species for ITS primers. Treatment was considered as a predictor variable in the analysis. We conducted pairwise comparisons with estimated marginal means (“emmeans” function and package; Lenth et al., 2023) using Sidak’s HSD test for GLM data.

The effect of taxonomy on network metrics was assessed by fitting linear regression models for each amplicon pair data set, ITS2+V1–V2 and ITS2+V3–V4. A t-test was performed on the estimated values to detect taxa that significantly explained the results of the network metrics.

All statistical analyses were performed with R software (version 4.2.2) (R Core Team, 2023).

TABLE 2 Primers used in this study.

Specificity	Region	Forward Primer (5'–3')	Reverse Primer (5'–3')	References
Fungi	ITS1–ITS2	CTTGGTCATTTAGAGGAAGTAA	GCTGCGTCTTCATCGATGC	Manter and Vivanco (2007)
Bacteria	16S V1–V2	GAGTTTGATCMTGGCTCAG	GCTGCCTCCCGTAGGAGT	Tuner et al. (1999)
Bacteria	16S V3–V4	CCTACGGGNGGCWGCAG	GACTACHVGGGTATCTAATCC	Herlemann et al. (2011)

3 Results

3.1 Chitosan reduced potted soil pH conductivity and cation exchange capacity but not under field conditions

Chitosan solutions significantly reduced soil pH (ANOVA; p value=0.001) (Figures 2A, B; Supplementary Table S1) and electrical conductivity (EC) (ANOVA; p value=0.04) (Figures 2C, D, Supplementary Table S3) when water content was maintained at field capacity in the pot experiment. Both chitosan solutions and coacervates reduced soil cation exchange capacity (CEC) with respect

to controls in the pot experiment (ANOVA; p value=0.03) (Figures 2E, F, Supplementary Table S5). However, under field conditions when applied monthly, chitosan did not alter field soil pH, EC, and CEC (ANOVA; p value = 0.5, p value= 0.3, p value= 0.1; Figures 2B, D, F; Supplementary Tables S2, S4, S6). In the field experiment, soil EC was lower for March–July than for November–February recordings for both soil managements (Supplementary Figure S1). In June and July, in the organic soil, conductivity could not be recorded because of low soil humidity for high temperatures and low rainfall (Supplementary Figures S2, S3). Chitosan application to field soil had no significant effect on soil mineral nitrogen content (Supplementary Figure S4).

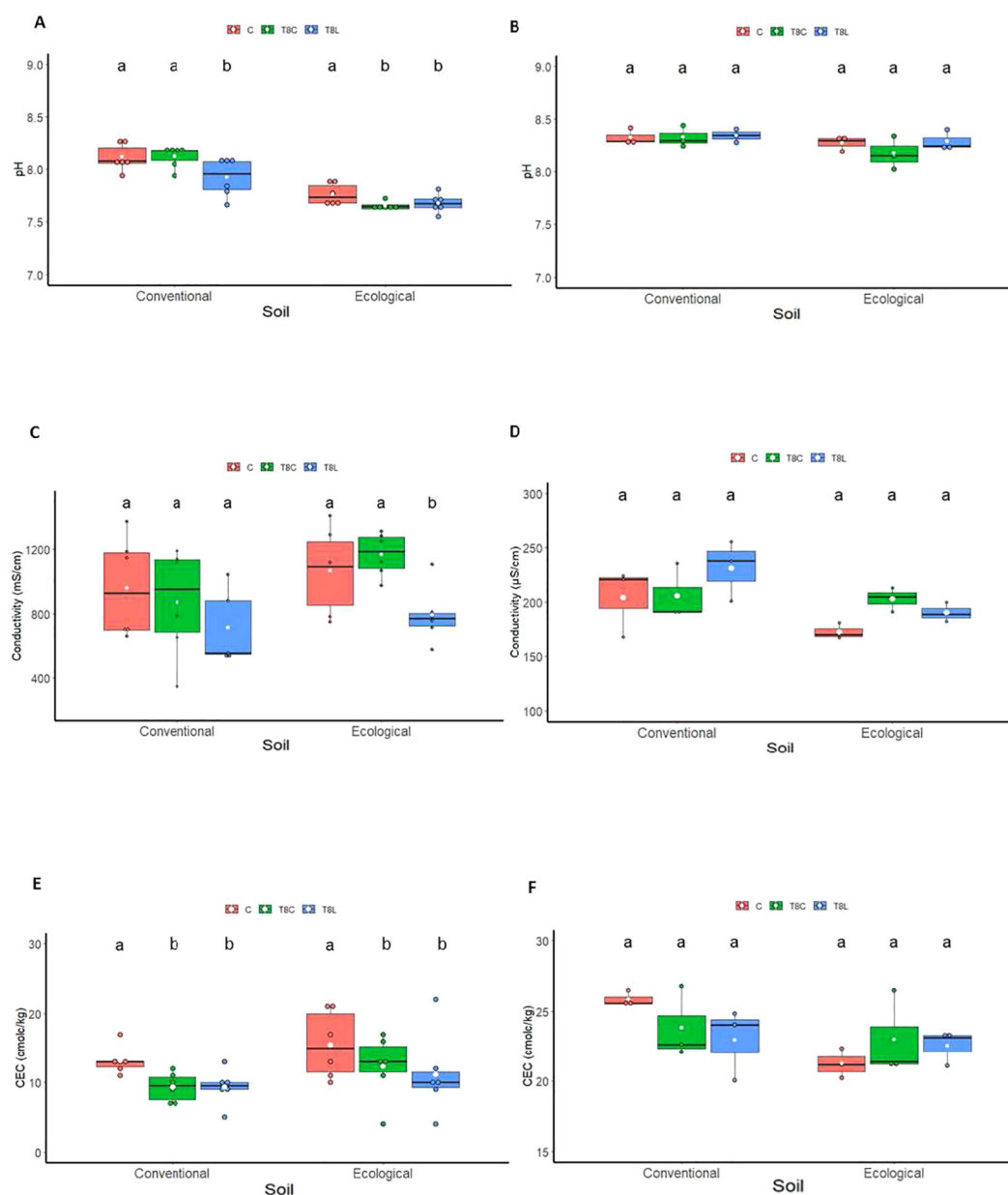


FIGURE 2

Effect of chitosan on soil chemical properties: soil pH (A, B), conductivity (C, D) and cation exchange capacity (E, F). Treatments: control [(C), untreated], chitosan coacervates (T8C), and chitosan solution (T8L). Experiments: growth chambers (A, C, E), field (B, D, F). Lowercase letters indicate significant differences between treatments for each soil type.

3.2 Chitosan did not affect field soil respiration under field conditions

A trend of increased respiration was observed in the chitosan treatments mainly from March to June for the conventional field soil (Figure 3A), and from March and May for the ecological field soil (Figure 3B). Irrespective of treatments, field soil respiration significantly (ANOVA; p -value > 0.001, Supplementary Table S7) increased in both management regimes (conventional and ecological) from March until July. This period corresponds with a steady significant increase in soil temperature for both conventional and ecological regimes (Supplementary Figures S2A, B). Soil moisture increased in the March recording (Supplementary Figures S2C, D). This corresponded, in turn, with an increase in precipitation and temperature (Supplementary Figure S3).

3.3 Chitosan modified soil mycobiota by reducing naturally occurring plant pathogenic fungi in soil under field conditions

Chitosan significantly (multivariate GLM, p value 0.001, Supplementary Table S8) modified fungal genera composition of ecological field soil (Figures 4A, B, 5A). Conversely, chitosan caused no significant differences in bacterial taxa composition of the same soil respect to untreated controls (multivariate GLM, p value > 0.001; Figure 5B). The fungus *Fusarium* was the fungal genus most present (33%–23%) in field samples (Figure 5A), followed by *Lachnellula* (22%–13%), *Wickerhamiella* (17%–14%), and *Filobasidium* (11%–7%) (Supplementary Table S9). Other genera, including *Alternaria*, showed 5% or less relative abundance (Figure 5A). Chitosan coacervates tended to reduce the relative abundance of *Fusarium* and *Alternaria*, although no significant differences were found. Presence of the plant pathogenic species *Fusarium falciforme* (50% reduction, Supplementary Table S10) in soil was significantly reduced (univariate GLM, p -value = 0.03, Supplementary Table S11), by chitosan solution (Figure 4B).

Chitosan coacervates significantly reduced (univariate GLM, p value = 0.01, Supplementary Table S11) the relative abundance of the phytopathogenic species *Alternaria atra* (20% reduction, Supplementary Table S10), with respect to untreated controls.

3.4 Chitosan coacervates increased naturally occurring nematophagous fungus *Purpureocillium* in soil under field conditions

Chitosan coacervates significantly (univariate GLM, p value = 0.006, Supplementary Table S12) increased (ca. 50-fold) naturally occurring nematophagous fungus *Purpureocillium* in field soil (Figure 5A). Significant differences were found for the variable fungal species relative abundance (multivariate GLM, p value = 0.044; Supplementary Figure S5, Supplementary Table S13) between control and chitosan coacervate treatments. Chitosan coacervates significantly increase (ca. 3,500%) the presence of the invertebrate pathogen *Purpureocillium takamizusanense* in soil (univariate GLM, p = 0.006, Figure 5B; Supplementary Table S11).

3.5 Nematophagous fungi and structure of soil microbiota

The use of ITS2+V1–V2 and ITS2+V3–V4 regions revealed variations in the co-occurrence network outcomes (Figure 6; Supplementary Tables S14, S15). However, we showed that the weighted degree centrality (WDC) parameter could not allow to detect contrasting influence of microbial groups within the network of each amplified region in the ITS2+V3–V4 subset, only marginally detected in bacteria that belonged to *Acidimicrobiales* (Figure 6A).

The clustering coefficient (CC) parameter for ITS2+V1–V2 regions showed that the nematode egg-parasitic fungi *Pochonia* (CC = 0.22) promoting network clustering into modules (n = 15 modules) (Figure 6B; Supplementary Table S16). By evaluating the

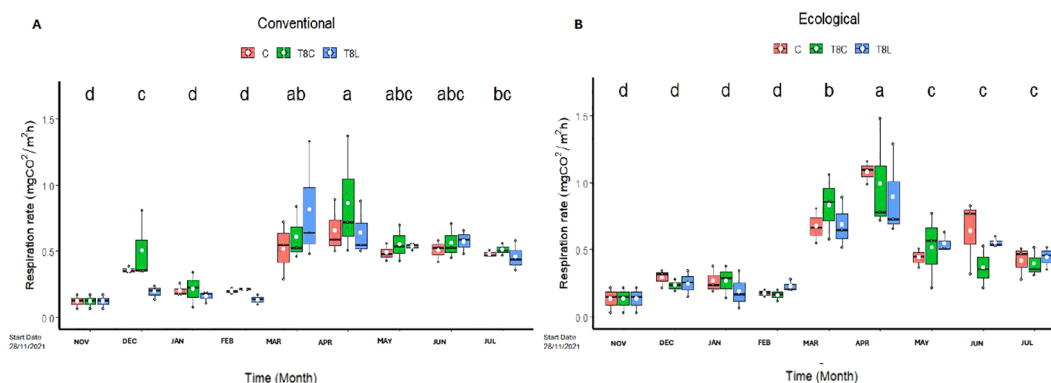


FIGURE 3

Effect of chitosan on field soil respiration. Soil was under conventional (A), or ecological (B) regimes. Treatments: field (C, untreated), chitosan coacervates (T8C), and chitosan solution (T8L). Lowercase letters show significant differences between the different times. Level of significant differences p -value < 0.05.

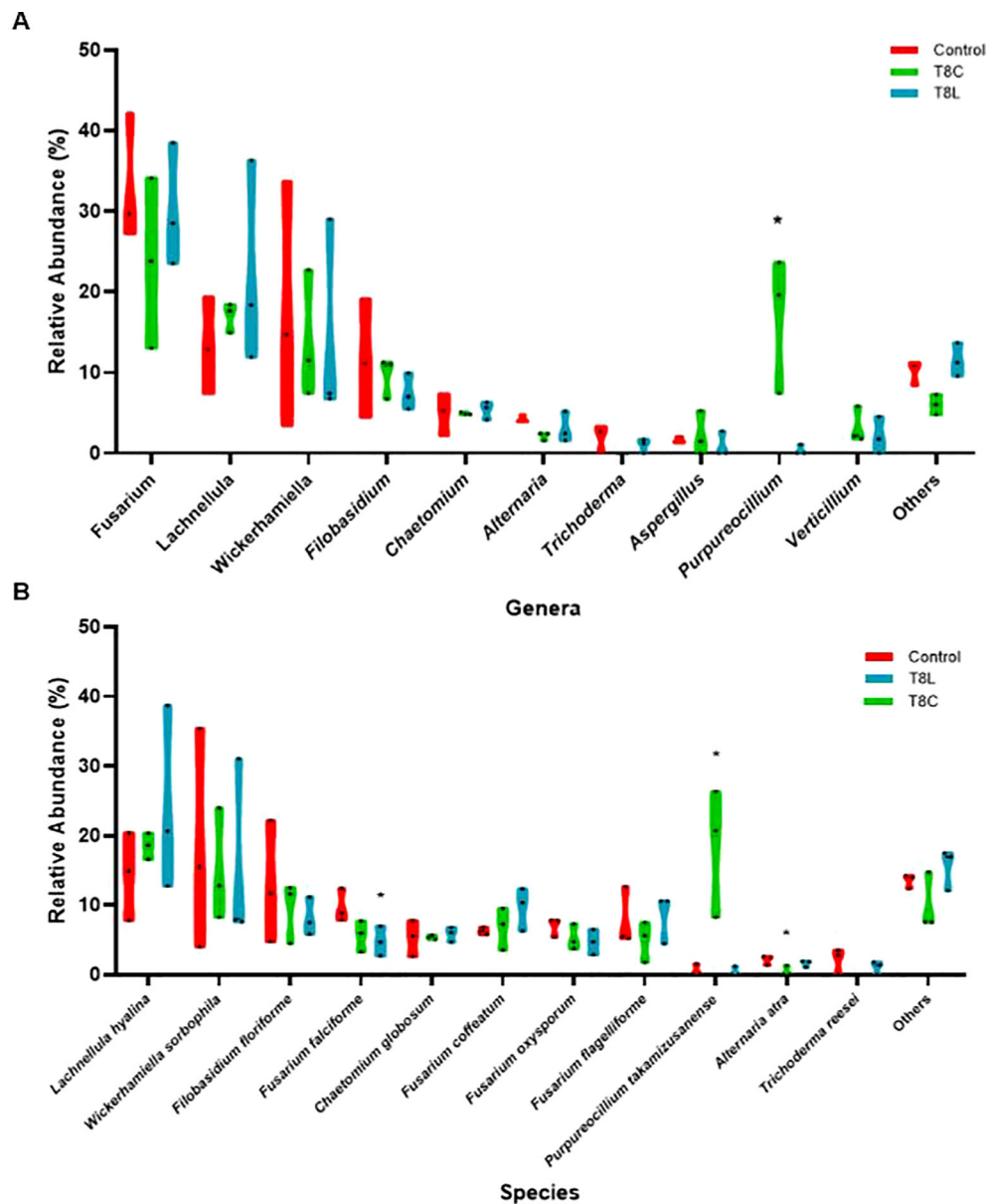


FIGURE 4

Relative abundance (%). Asterisks mark significant differences (p value < 0.005), of the treatments with respect to the control for each genus (A) and species (B).

co-occurrence results, we detected *Pochonia chlamydosporia* with a positive interaction to xylan-degrading (*Humisphaera*), N-fixing (*Leptolyngbya*), and sulphate-reducing bacteria (*Rubrobacter*) (Supplementary Table S18). Furthermore, we detected antagonistic interactions with soil bacteria such as *Aquihabitans* spp., a Gram-negative bacteria, *Leptolyngbya* spp., a worldwide distributed cyanobacteria, and *Proteatibacter* spp., a widely distributed soil bacteria (Supplementary Table S18). The ITS2+V3–V4 regions showed that the nematode-trapping fungi *Orbilia* ($CC = 0.20$) and the Order *Acidimicrobiales* ($CC = 0.17 \pm 0.02$) and *Cytophagales* ($CC = 0.04 \pm 0.01$) significantly contributed to network clustering into modules ($n = 29$) (Figure 5B; Supplementary Table S17). *Orbilia oligospora* showed synergistic co-occurrence with a wide group of soil bacteria (*Nakamurella* spp., *Nocardioides* spp., or *Vulgatibacter* spp.).

By the other side, *O. oligospora* showed a competitive behaviour with important soil borne fungal pathogens like *Talaromyces* spp. and *Aspergillus* spp. species (Supplementary Table S19).

Acidimicrobium ferrooxidans, an extremophile bacteria able to grow under extremely low-pH conditions ($pH < 2$), showed positive interactions with soil-living bacteria such as *Massilia* spp., *Nitrospira* spp., or *Stella* spp. However, this bacterium had an antagonistic effect on *Jiangella* spp., *Hymenobacter* spp., and *Limnoglobus* spp. bacteria present in crop soils. Inside of the *Cytophagales*, the species *Cytophaga hutchinsonii* showed positive interactions with many soil-born bacteria (*Calothrix* spp., *Chitinophaga* spp., or *Lysobacter* spp.). Furthermore, *C. hutchinsonii* revealed negative associations with important soil fungal pathogens like *Fusarium oxysporum* and *Verticillium dahliae*.

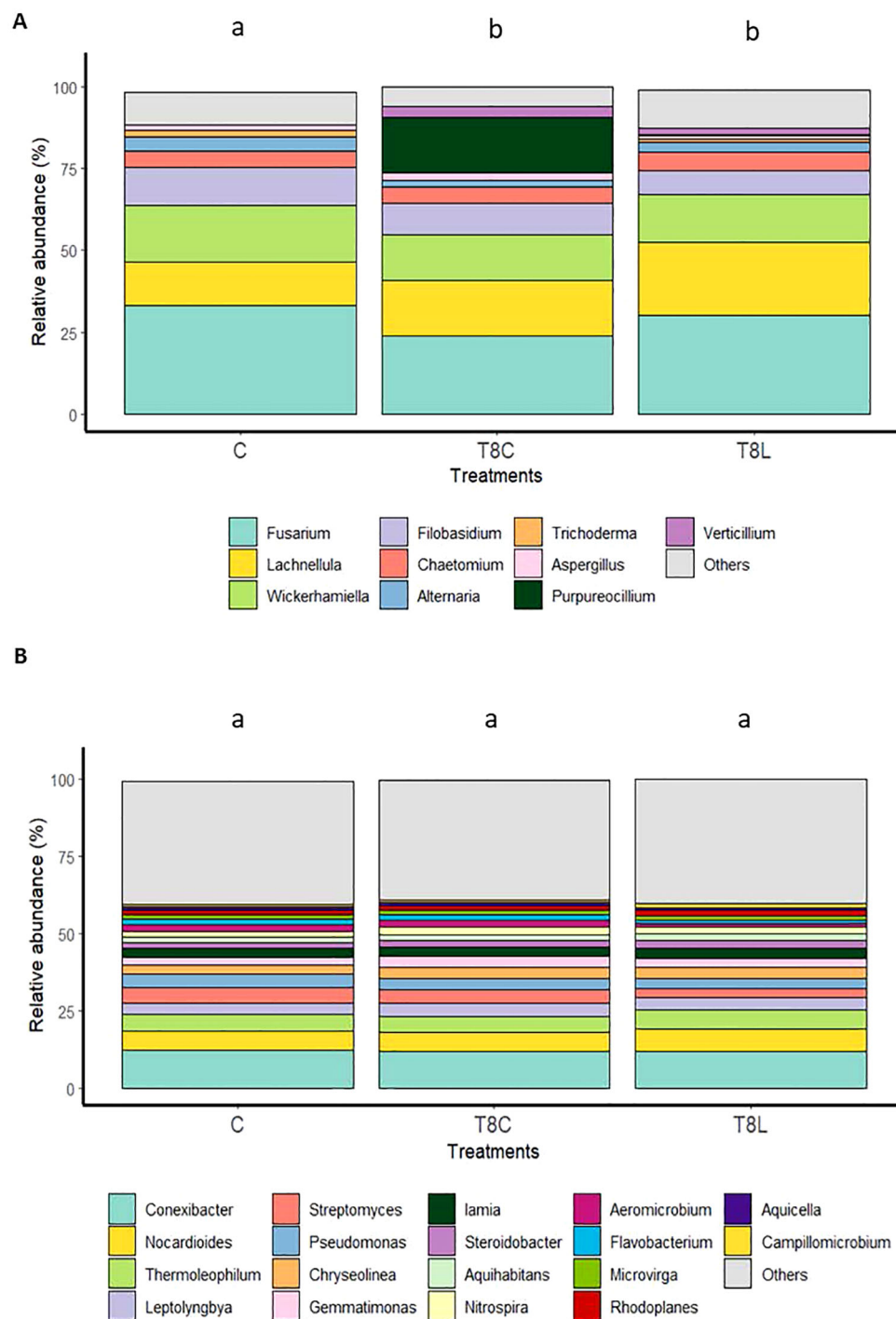


FIGURE 5

Effect of chitosan on field soil microbiota. (A) Fungal genera (ITS primers) and (B) bacterial genera (V1–V2 primers). Treatments: field (C, untreated), chitosan coacervates (T8C), and chitosan solution (T8L). Different letters indicate significant differences (p -value < 0.05).

4 Discussion

Chitosan applied maintaining soil water content at field capacity in pots for a month significantly reduced soil pH, CE, and CEC. The slight reduction of pH in the soil induced by chitosan could be simply due to the weak acidity of chitosan solutions. This effect was not found under field conditions. This was perhaps by the lower volumes of chitosan

solutions applied monthly. The high calcium carbonate content of both soils could neutralise the chitosan solutions. The reduction of soil CE by chitosan in pots could be associated with the mopping capacity of chitosan (polycation) of ions present in the soil solution (Kamari et al., 2011). Chitosan solutions and coacervates reduced soil cation exchange capacity (CEC) with respect to controls for potted soils. For example, when applied to sodium montmorillonite, chitosan intercalates in the

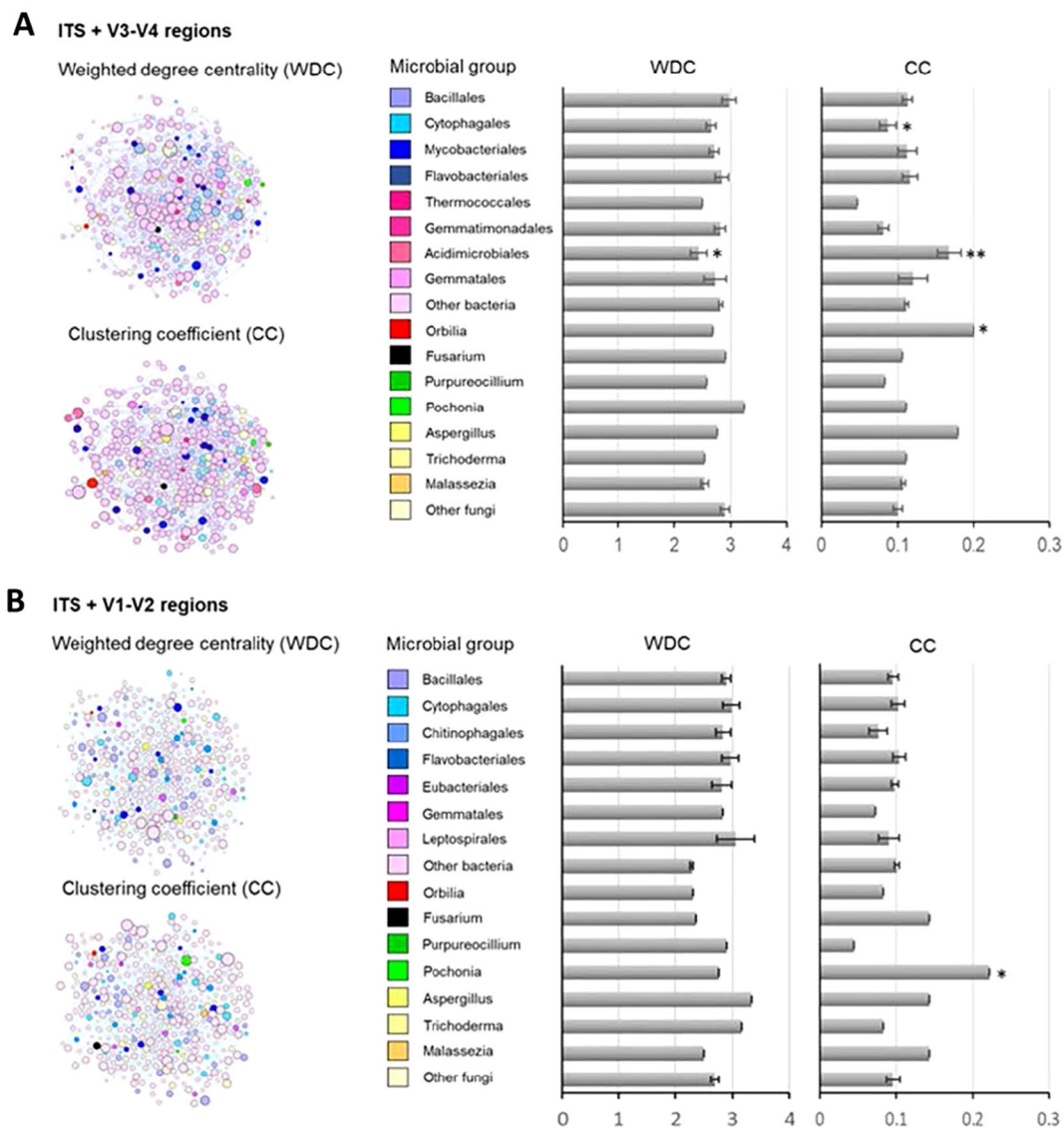


FIGURE 6

Microbial co-occurrence networks by amplified region pairs, ITS (fungi) and V1–V2 (A) / V3–V4 (B) (bacteria) in soils. Networks were inferred from SPIEC.EASI R package. Graphs and network metric-weighted degree centrality (WDC), i.e., the interaction frequency between OTU pairs, and clustering coefficient (CC), i.e., the extent of an OTU to cluster with others into modules, were determined in Gephi software. WDC and CC values are also shown in bar graphs by microbial group, bacteria, and fungi at order and genus taxonomic levels, respectively. The effects of microbial taxa identity on the network metrics were evaluated by linear models and t-tested, and those significantly explaining WDC and CC are marked by the significance level: ** $p < 0.01$, * $p < 0.05$.

layers of the clay (Darder et al., 2003), both reducing the negative charges for cation exchange and immobilising chitosan in soils. In our pot study, applying this chitosan may have displaced exchangeable cations from the clay complex, thus reducing CEC. However, this was not found when chitosan was applied monthly in the field. The regime of chitosan irrigation (field capacity vs. monthly applications) could account for a lower chitosan presence in field soil than in the pots. This may have made the chitosan displacement of cations of the clay complex in field soil less efficient than in pots. Taken together, our results suggest that chitosan can be applied to agricultural fields without affecting CEC, a key parameter for soil fertility (Anderson et al., 2023).

Undissolved chitosan added to soil (5% w/w) caused N increase (ammonium and nitrogen), with respect to untreated controls in previous microcosm experiments (Sawaguchi et al., 2015). In our study, chitosan application to field soil had no significant effect on soil mineral nitrogen content due mainly to the high mobility of mineral in soils. Our treatments also involved less chitosan applied to soil than in the microcosm. This, and the time lapse (9 months) for N soil content testing, may explain our results. In soil incubation experiments with chitosan, soil respiration was found to increase with chitosan concentration (Nkoh et al., 2024). In our field study, chitosan treatments resulted in increases in soil respiration, especially during spring–midsummer. This effect, although not

significant, could be related to eventual organic N input when chitosan was added to our microplots.

Our metabarcoding study shows that chitosan significantly modifies fungal genera composition of ecological field soil. Chitosan coacervates increase naturally occurring nematophagous fungus *Purpureocillium* in soil with respect to chitosan solution treated soil and untreated controls. Chitosan increases by ca. 6,000% conidiation of *Purpureocillium* (Palma-Guerrero et al., 2010c) cultures with respect to control media with no chitosan. The similar increase (ca. 50-fold) in the relative abundance of *Purpureocillium* spp. found in this work could be due to chitosan induction for conidiation of the fungi naturally occurring in soil. The highly sporulating capacity of this chitosan-tolerant fungal genus could explain our results (Gortari and Hour, 2016). Indeed, *Purpureocillium lilacinum* was previously applied combined with chitosan promoting managing effects on root knot nematodes (Giannakou et al., 2020; Zhan et al., 2021). In our study, we find the species *Purpureocillium takamizusanense*, which has been also isolated as an entomopathogenic fungus (Nguyen et al., 2022). Future studies should evaluate the effect of chitosan on the performance of this fungus in the field for insect/nematode pest biomanagement. These studies should include augmentative natural biocontrol and enhanced biocontrol with inundative or sustained additions of inoculum of the fungus. Furthermore, chitosan particles should be used in these studies, since chitosan solutions did not enhance naturally occurring *Purpureocillium* on soil.

We also found that the abundance of *Alternaria atra* and *Fusarium falciforme* decreased in soil treated with a chitosan solution with respect to control soil. Chitosan accumulates in the cell wall of non-chitinolytic fungi, thus preventing their growth (Muzzarelli et al., 1986). However, the plasma membrane is the main target of chitosan (Lopez-Moya et al., 2019). Chitosan-sensitive fungi, e.g., *Fusarium*, have fluid membranes with respect to chitosan-resistant fungi such as *Purpureocillium*. These two fungal species cause diseases in several crops worldwide (Bonthala et al., 2021; Trolinger et al., 2017). Therefore, soil treatment with chitosan could be a sustainable alternative for managing these fungal plant pathogens. Furthermore, our co-occurrence network analyses show that *Purpureocillium* spp. negatively related to *Alternaria atra* and *A. rosae* (Supplementary Table S18). *Purpureocillium* spp. are fungi well known to produce antimicrobial secondary metabolites (Chen and Hu, 2021). Future studies should investigate the mechanisms involved in the antagonism of *Purpureocillium* spp. to *Alternaria* spp. in soil.

Metagenomics on soil exposed to chitin-rich exoskeletons has been a source of gene sequences encoding chitin–chitosan-degrading enzymes (Li et al., 2015; Stöveken et al., 2015). Most of these sequences were of bacterial origin. Our metabarcoding analysis shows that chitosan application during 9 months to field soil did not change bacterial taxa profiles. Perhaps time of exposure to chitin/chitosan could account for these differences.

We have carried out a microbial diversity and ecological network analysis (Barberán et al., 2012). Our results show that the two main ecological groups of nematode-destroying fungi (Barron, 1997), nematode trapping (*Orbilia* spp.) and egg parasites (*Pochonia* spp.), promote soil microbe network clustering into modules.

Nematophagous fungi interact with nematodes, the most abundant animal taxon in soil (Dervash et al., 2018). Since most soil nematodes are bacterivorous (De Mesel et al., 2004), it was expected that nematophagous fungi were also related to soil bacteria. Indeed, our co-occurrence network analyses show that the nematode egg parasite fungus *Pochonia* positively related to xylan-degrading (*Humisphaera*), N-fixing (*Leptolyngbya*), and sulphate-reducing bacteria (*Rubrobacter*). These soil prokaryotes could help with nutrient acquisition by the fungus. *Nocardioide*s, a hydrocarbon degrader, antibiofilm and antibiotic producer filamentous bacterium, is negatively correlated with *Pochonia* and positively with *Orbilia*. This and other bacteria (*Paraflavitalea*, Chitinophagaceae), also positively related with *Orbilia*, can degrade chitin in soil. Root nodule bacteria (*Microvirga* and *Botea*) are positively correlated with the nematode trapping fungus. *Pochonia* can show endophytic lifestyle in crop plants and can be beneficial for plant defence against soil-borne pathogens (Manzanilla-Lopez et al., 2013). Nematode egg fungal parasites are multitrophic organisms than can be enhanced by chitosan (Escudero et al., 2016; 2017). In this work, we find that chitosan application in soil enhances *P. lilacinum* recruitment and the promotion of *P. chlamydosporia* as key fungi to structure microbial communities in soil. Bacteria belonging to *Acidimicrobiales* and *Cytophagales* also significantly contributed to network clustering in field soil. These groups are documented to act on iron redox-related processes (Garber et al., 2021) and carbohydrate polymer (chitin, pectin, cellulose) turnover (Mohapatra et al., 2022) in soil, respectively. They play a key role in recruiting soil-borne bacteria essential to maintaining soil health. In addition, we show that *C. hutchinsonii* is an antagonistic microorganism against two important plant pathogenic fungi such as *V. dahliae* and *F. oxysporum* (Kausar et al., 2021).

In conclusion, this work has shown that chitosan in the form of coacervates increases the abundance of *Purpureocillium* in soil (ca. 50-fold). Nematophagous fungi, both egg parasites (*Pochonia*) and predatory (*Orbilia*), promoted soil microbiota network clustering.

Future studies could combine the use of these fungi with chitosan to treat diseases in various agricultural crops. Our work opens new and promising possibilities to develop integrated strategies based on the use of chitosan formulations to improve soil health and for managing important plant diseases caused by plant parasitic nematodes.

Data availability statement

The datasets presented in this study can be found in online repositories. The data presented in the study are deposited in the NCBI repository, accession number PRJNA1164777 (<https://www.ncbi.nlm.nih.gov/sra/PRJNA1164777>). The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Author contributions

RL-N: Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. JP-R: Data curation,

Formal analysis, Investigation, Software, Writing – original draft, Writing – review & editing. IB: Conceptualization, Formal analysis, Investigation, Methodology, Validation, Writing – original draft, Writing – review & editing. AL: Conceptualization, Investigation, Methodology, Supervision, Visualization, Writing – original draft, Writing – review & editing. MVU: Data curation, Formal analysis, Methodology, Software, Validation, Writing – original draft, Writing – review & editing. FL-M: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. LL-L: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This research was funded by the PID2020-119734RB-I00 Project from the Spanish Ministry of Science.

Acknowledgments

We would like to thank C.R.A, Agustin Chumillas Roldán, from the Plant Pathology Laboratory (University of Alicante) for his technical support.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fagro.2024.1502402/full#supplementary-material>

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OPEN ACCESS

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RECEIVED 04 October 2024

ACCEPTED 29 November 2024

PUBLISHED 30 January 2025

CITATION

Ng'ang'a SK, Ogutu SO, Tibebe D, Akinyi D
and Mockshell J (2025) Comparative
profitability of agroecological
practices in Ethiopian wheat farming.
Front. Agron. 6:1502786.
doi: 10.3389/fagro.2024.1502786

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Comparative profitability of agroecological practices in Ethiopian wheat farming

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Introduction: Agroecology is increasingly promoted as a pathway to sustainable food production, aiming to maximize natural resource use while minimizing external inputs with harmful environmental effects. Agroecological practices can enhance farm productivity while ensuring environmental sustainability. However, these practices often require higher initial investments compared to business-as-usual (BAU) practices, and their profitability and relative risks are not well studied. This research evaluates the profitability and risk of adopting agroecological practices among wheat farmers in Ethiopia.

Methods: We conducted a deterministic cost-benefit analysis (CBA) incorporating sensitivity and scenario analysis to evaluate the profitability and relative risks associated with three agroecological practices: certified wheat seed, optimal site-specific inorganic fertilizer application rates, and drainage of waterlogged soils. The analysis considered yield uncertainty, market price fluctuations, and implementation variability to provide robust insights for decision-making.

Results: The deterministic CBA revealed that among the three practices, the use of certified seeds was the most profitable, with a net present value (NPV) of US\$ 2,531 ha⁻¹. This was followed by optimal site-specific inorganic fertilizer application, with an NPV of US\$ 2,371 ha⁻¹. Drainage of waterlogged soils yielded the lowest profitability, with an NPV of US\$ 2,099 ha⁻¹.

Discussion: The results indicate that certified seeds and optimal fertilizer rates offer higher financial returns, making them attractive investments for wheat farmers. However, profitability alone does not guarantee adoption. Other factors, including social and behavioral aspects, influence farmer decisions. Future research should integrate these dimensions to develop comprehensive strategies for promoting agroecological practices.

Conclusion: Adopting agroecological practices has clear economic benefits for Ethiopian wheat farmers, with certified seeds emerging as the most profitable option. These findings provide evidence for stakeholders to design targeted interventions that maximize returns while addressing barriers to adoption.

KEYWORDS

agroecology, innovations, Ethiopia, cost-benefit, wheat, farmers

1 Introduction

Global food systems are at a critical juncture, grappling with unprecedented challenges in providing healthy, accessible diets to all people while safeguarding environmental health (Herrero et al., 2021). These challenges are compounded by hunger, malnutrition, climate change, resource depletion, biodiversity loss, and economic instability, all of which directly threaten farmers' livelihoods, and rural development (Fan et al., 2021; Ewert et al., 2023; Mockshell and Kamanda, 2018). Recent crises like the COVID-19 pandemic and the Russia-Ukraine conflict, have further exposed the vulnerabilities within agri-food systems, disrupting supply chains, escalating food prices, and undermining global food security (Ewert et al., 2023; Mockshell and Nielsen Ritter, 2024). Addressing these interlinked issues requires not only innovation in agricultural production but a paradigm shift toward more sustainable, resilient food systems (Piñeiro et al., 2020; Fan et al., 2021).

While much of the literature has underscored the need for such transformations, there remains a notable gap in understanding the financial viability and risk dynamics of transitioning to sustainable practices, particularly for smallholder farmers in sub-Saharan Africa. This study uniquely addresses this gap by focusing on the cost-benefit and risk analysis of specific agroecological practices within the wheat value chain in Ethiopia—a region that is underexplored in this context. Agroecology, recognized as a promising framework for achieving sustainable food systems, incorporates ecological principles to optimize interactions between farming components (Jones et al., 2022). This is because it aims to maximize the use of natural resources and minimize the reliance on external inputs, promoting long-term productivity and environmental sustainability (Wezel et al., 2020). Examples of agroecological practices include using certified local seed (which can be open-pollinated-varieties that promote biodiversity), applying fertilizers at optimal rates, improving drainage in waterlogged soils, rotating crops, and embracing crop and farm diversity, planting cover crops, no-till systems, integrated pest management, and agroforestry practices (Piñeiro et al., 2020). However, there is limited empirical evidence on the profitability and relative risks of such practices, especially under smallholder farming conditions, where resource constraints and market access challenges further complicate decision-making.

In this paper, we take a novel approach by conducting a detailed cost-benefit analysis (CBA) coupled with sensitivity analysis to evaluate three specific agroecological practices prioritized by Ethiopian wheat value chain stakeholders: certified seeds, optimal site-specific fertilizer application, and waterlogged soil drainage. Contrary to business as usual (BAU) scenarios where farmers often engage in their day-to-day farming practices e.g., without using certified seeds, optimal site-specific inorganic fertilizer application rate, and draining waterlogged soils, the use of such agroecological practices could improve soil drainage, soil nutrient availability, agricultural productivity, and profits (Ali et al., 2015; Ayalew et al., 2022; Pais et al., 2023). These agroecological practices can, therefore, play a critical role in protecting the ecosystem by ensuring more efficient use of natural resources and strengthening the capacity to adapt to

climate change, resilience and environmental sustainability (Negra et al., 2020; Jones et al., 2022). However, the uptake of agroecological practices among smallholders in sub-Saharan Africa is still very limited, constrained by factors such as high initial investment costs, limited access to technology and information, labor demands, market access and potential trade-offs between maximizing productivity in the short term and achieving long-term sustainability and environmental protection (Akinyi et al., 2022; Mockshell and Kamanda, 2018). Additionally, some practices often associated with agroecology, such as the use of certified seeds and optimal fertilizer application, can be complex and require specific knowledge, which can further limit adoption by smallholder farmers.

Despite efforts to promote the adoption of agroecological and other sustainable agricultural practices, existing literature and climate adaptation programs have rarely examined the profitability and relative risk surrounding the practices (Akinyi et al., 2022; Mogaka et al., 2022). To help address this research gap, we evaluate the profitability (costs and benefits) and the relative risk through sensitivity analysis associated with three agroecological practices (certified seed, optimal site-specific inorganic fertilizer application rate, and drainage of waterlogged soils) among smallholder wheat farmers in Ethiopia. Sensitivity analysis, in particular, is a key innovative aspect of this study. It allows us to systematically assess how variation in critical parameters—such as input costs, crop yields, and climatic conditions—affect the profitability and risk of adopting these agroecological practices. This approach not only enhances robustness of our finding but also provides nuanced insights into how these practices might perform under different scenarios, which is crucial for smallholder farmers facing a range of uncertainties.

Unlike many existing studies that focus broadly on sustainability or productivity, our work delves into financial and risks-related dimensions of adopting these practices, providing crucial insights for smallholder farmers, policymakers and investors. By incorporating sensitivity analysis, we address a significant gap in the literature, offering a more dynamic understanding of how these practices might impact farm-level economics and risks profiles in varying conditions.

We conduct this study in Ethiopia because Ethiopia, like most other countries in sub-Saharan Africa, is affected by hunger and malnutrition, loss of biodiversity, conflicts, and climate change-related problems (FAO et al., 2022). Efforts to increase crop production in Ethiopia have recognized the importance of agroecology and implementing programs (e.g., the national soil and water conservation program, the sustainable land management program) and practices (e.g., conservation tillage, drought-tolerant varieties, and site-specific wheat varieties) that aim at ensuring sustainable production (Schmidt and Tadesse, 2019; Tanto and Laekemariam, 2019; Desta et al., 2021; Belete et al., 2022). Ethiopia, also, presents a particularly compelling case for this analysis due to its critical role in wheat production within Africa, coupled with its ongoing struggle against food insecurity, climate-related stressors and soil degradation (FAO et al., 2022; Nigus et al., 2022). As the second-largest producer of wheat in the continent, Ethiopia's ability to sustain and enhance wheat production has significant

implication for both national and regional food security. Despite this, wheat farmers in Ethiopia face systemic challenges, including limited access to improved seed varieties and degrading soils, which agroecological practices could help to mitigate (Anteneh and Asrat, 2020; Desta et al., 2021). By focusing on wheat, this study not only addresses a critical agricultural sector but also contributes to a broader understanding of how agro-ecological principles can be scaled in context that are vital to food security.

The remainder of this paper is structured as follows: Section 2, details the study area, data collection methodology, and CBA framework. Section 3 presents the results of our analysis, followed by a discussion of key findings, policy implications, and conclusions in Section 4.

2 Materials and methods

2.1 Study sites

The study area comprises three districts in Ethiopia: Goba, Lemo, and Munesa. Goba district is in Bale zone, Oromia Regional State of Ethiopia. It lies between 5°57'30"N to 7°12'00"N latitude and 39°35'00"E to 40°15'00"E longitude (Assefa et al., 2024). Its altitude ranges from 2400 to 4377 meters above sea level (masl). It has a total area of 1,674 km², and is located 445 km away from Addis Ababa, the capital city of Ethiopia (Legesse et al., 2019). Its monthly temperature ranges from 4°C to 25°C, and annual rainfall varies from 900 mm in the lowlands to 1,400 mm in highlands (Assefa et al., 2024). Agriculture is the most dominant economic activity in the district, with cereals (including wheat), horse beans, field beans and lentils being the most important crops grown (Legesse et al., 2019).

Lemo district is one of the districts in the Hadiya zone of southern Ethiopia. It lies between 7° 24' 0''N and 7° 44' 30''N latitude and 37° 44' 0''E and 38° 3' 0''E longitude (Sedebo et al., 2021). Its altitude ranges from 1500 to 2500 masl (Tadesse et al., 2014). It has a total area of 34,986 ha (Sedebo et al., 2021), and is located about 230 km southwest of Addis Ababa (Addise et al., 2022). Its mean annual temperature ranges from 15 to 22°C and rainfall ranges from 700 to 1,260 mm (Sedebo et al., 2021). Cereals are the most cultivated crops in the area, accounting for about 60% of all crop production. Wheat is the most dominant cash crop produced in the district (Sedebo et al., 2021).

Munesa district is located in the East Arsi zone of Oromia region, Ethiopia. The district lies between latitudes 7°12' to 45° N and longitude 52° to 39°03'E in central Ethiopia (Adunee and Fekadu, 2019). Munesa is located 232 km southwest of Addis Ababa. Its altitude ranges from 2080 to 3700 masl and is characterized by mid sub-tropical temperature ranging from 5 to 20°C. The total land area covered by the district is 1031 km² with a total population of 211,762 (Adunee and Fekadu, 2019). Crop-livestock integration is the dominant farming system within the district. Major cereal crops cultivated include wheat, barley, and maize (Adunee and Fekadu, 2019).

2.2 Prioritization of agroecological innovations/practices

The CCAFS-CSA Prioritization framework (FAO, 2010; Corner-Dolloff, 2014) was adopted and customized to identify and prioritize agroecology practices in this study. This framework guides stakeholders through the process to filter a long list of applicable agroecology practices into prioritized ones (Khatri-Chhetri et al., 2017; Thornton et al., 2017). According to Corner-Dolloff (2014), the approach involves three major phases:

- i. Compilation and assessment: Collecting a long list of agroecology practices and assess/characterize them based on FAO's 10 elements of agroecology indicators.
- ii. Prioritization: Identifying and shortlisting top agroecology practices based on scores.
- iii. Cost-benefit analysis: Conducting cost-benefit analysis of the selected agroecology practices.

The identification and prioritization for wheat value chain were conducted during a workshop by involving 20 participants. These participants included district-level agricultural experts and cooperative representatives from Munesa and Goba districts of Oromia region, and Lemo district of central south region of Ethiopia, agricultural researchers from Ethiopian Institute of Agricultural Research, federal experts from Ministry of Agriculture, agroecology practitioners from NGOs and Civic society, and researchers from Haramaya University and Alliance of Bioversity International CIAT. Participants were divided into three groups representing the three districts considered in this analysis. The workshop employed the customized CCAFS CSA prioritization framework (Lizarazo et al., 2021; Mwongera et al., 2018) using the following steps:

1. Identification: Participants identified 13 agroecology practices implemented in wheat production system in Ethiopia based on their knowledge and literature. The practices identified were certified wheat seeds, site specific optimal fertilizer, drainage-BBF with wheat, crop rotation with leguminous and oil crops, agroclimate advisory, integrated pest management, crop residues, organic amendment-compost, agroforestry, green manuring during off season, fallow, optimal irrigation and farmyard manure.
2. Evaluation: The FAO's 10 agroecology elements (diversity, co-creation and sharing of knowledge, synergies, efficiency, recycling, resilience, human and social values, culture and food traditions, responsible governance, circular and solidarity economy) were used as indicators to evaluate the practices.
3. Scoring: Participants scored the 13 agroecology practices against the 10 agroecology elements using Likert scale from -3 to 3: high positive effect, 2: medium positive effect, 1: low

positive effect, 0: no effect, -1: low negative effect, -2: medium negative effect, -3: high negative effect). Scoring was done through discussion and consensus.

4. Aggregation: The Likert score for each agroecology practice against the 10 elements were summed up and averaged.
5. Ranking: Practices were ranked based on the average scores. Results from each group were presented in a plenary for further discussion, review, cross-fertilization, and experience sharing among the group.
6. Discussion: The top three agroecology practices were described in detail.

2.3 Agroecological intervention

The purpose of this study is to estimate the net benefit of three prioritized agroecological innovations and to estimate the net impact of these innovations on the income. There is thus a need to first understand the revenues and expenditures of the activities adopted by the households in relation to these innovations. Next, one needs to compare the values with revenues and expenditures under the studied innovations. This comparison will allow one to evaluate whether the incremental benefit of innovations is worth the cost. This is carried out by building both a “with” and “without” scenario with respect to revenues and expenditures profiles. An incremental cash flows statement is then constructed for the entire evaluation period of thirty years.

2.3.1 “Without” intervention/“Business as Usual” scenario

2.3.1.1 Certified seed

In the absence of certified seeds, wheat farmers rely on traditional or uncertified seeds that often have lower germination rates and genetic purity. This results in inconsistent yields, higher susceptibility to pests and diseases, and reduced resilience to environmental stresses (Baglan et al., 2020). The overall productivity is lower, leading to less marketable produce and reduced income for farmers. Soil health may also deteriorate over time as lower-quality seeds do not support robust plant growth (Rios et al., 2009).

2.3.1.2 Drainage systems

Without proper drainage systems, waterlogging can become a significant issue, especially during heavy rainfall. This can lead to root rot, reduced plant growth, and lower wheat yields (Iizumi et al., 2024). Poor drainage also exacerbates soil erosion and nutrient leaching, leading to long-term soil degradation (Motarjemi et al., 2023). The economic impact includes lower yields and quality, resulting in reduced market prices and income for farmers (Rios et al., 2009).

2.3.1.3 Without optimal fertilizer rates

Using non-optimal fertilizer rates—either too much or too little—can lead to several problems. Over-fertilization can cause nutrient runoff, pollution, and soil acidification, while under-fertilization

results in poor plant growth and lower yields (Caplan et al., 2017). Inefficient fertilizer use leads to wasted resources and additional costs without corresponding increases in productivity (Smil, 2004). This negatively impacts both the environment and farmer incomes due to reduced yield and quality.

2.3.2 “With” intervention scenario

2.3.2.1 Certified seed

Using certified seeds ensures high germination rates, genetic purity, and improved resistance to pests and diseases. This results in more consistent and higher yields, better-quality produce, and increased farmer income (Dhiman et al., 2010). Certified seeds also contribute to better soil health as they are often bred to be more efficient in nutrient uptake, reducing the need for excessive fertilizer application. The initial investment in certified seeds is offset by the increased productivity and market value of the crops.

2.3.2.2 Drainage systems

Implementing effective drainage systems helps prevent waterlogging, promoting healthier root systems and optimal plant growth (Iizumi et al., 2024). This leads to increased wheat yields and better-quality produce. Proper drainage also minimizes soil erosion and nutrient leaching, contributing to long-term soil fertility and sustainability (Harris et al., 2016). The initial costs of installing drainage systems are justified by the increased productivity and resilience of the agricultural land, ultimately enhancing farmer incomes and market competitiveness.

2.3.2.3 Optimal fertilizer rates

Applying optimal site-specific inorganic fertilizer rates ensures that plants receive the necessary nutrients for optimal growth, resulting in higher yields and better-quality wheat (Mesfin et al., 2021). This practice improves nutrient use efficiency, reducing the risk of environmental pollution from runoff and maintaining soil health (Wang et al., 2023). Farmers benefit economically from higher productivity and lower costs associated with overuse or underuse of fertilizers. The environmental impact is also positive, as optimized fertilizer use contributes to sustainable farming practices.

2.4 Data collection

The study used primary data collected in 2023 from key informants in three districts: Goba, Lemo, and Munesa. The key informants included stakeholders from the ministry of agriculture, universities, research institutes, farmer group representatives, and farmers. Key informants were purposively selected based on their experience with both “Business as Usual” (BAU) or “Without Intervention Scenario” and “With Intervention (i.e., agroecological practices, specifically “optimal site-specific inorganic fertilizer rate”, “certified seeds” and drainage in the wheat value chain) Scenario. Data collection was done using structured household questionnaires, which included qualitative variables (e.g., variables identifying and describing the agroecological practices adopted and the BAU case, variables describing reasons why agroecological practices are

preferred) and quantitative variables (e.g., on yield, prices of inputs and output, labor, and services costs). The questionnaire used to collect the data is provided in the [Appendix](#). Literature review was conducted to fill any potential data gaps, such as historical variations in yield, input prices, and discount rates. Sixteen key informant interviews were conducted. Eight of the interviews compared application of fertilizers at optimal rates with BAU practices. Six interviews compared the use of certified wheat seed with BAU practices, while two other interviews compared draining of water-logged soils with BAU practices.

2.5 Data

Two types of surveys were conducted for this study. The first survey aimed at collecting data about the innovations from the Key Informants. The data included details of the most common agroecological practices applied by wheat farmers in the study area. About 13 agroecological practices were identified by the key informants as the most widely practiced. A second survey focused on cost-benefit analysis (CBA) of the three innovations that were innovations. This survey captured cost data across three categories: implementation (machinery, equipment, labor, infrastructure), maintenance (lifespan), and activity (ongoing operational expenses). Refer to [Ng'ang'a et al. \(2021\)](#) for a detailed breakdown of these cost categories, and to [Appendix A](#) for the specific questions that were asked.

A before-and-after costing approach was used for data collection. Experts compared the innovation's installation, maintenance costs, and resulting yields to a baseline business-as-usual (BAU) scenario and the innovation (also referred to as agroecological practices). The experts provided detailed information on factors impacted by the innovation: installation, maintenance, operation costs; input demand (seeds, fertilizers); yield changes; and cost of capital. This involved itemizing all activities associated with the implementation (establishment), maintenance, and operations (post-harvesting activities) of the BAU and the innovations variable inputs, transportation costs, yield per hectare, and market prices for both BAU and the innovation. All data was then converted into monetary values.

Costs were categorized into production costs (labor for various tasks, equipment, services, variable inputs, transportation) and benefits (gains from the innovation, e.g., increased yield, reduced maintenance etc.).

The study utilized both primary and secondary data sources. Primary data came from the expert survey. Secondary data, primarily from peer-reviewed literature and country reports, filled any gaps in the primary data, such as historical variations in yield, input prices, and discount rates.

2.6 Analysis

Following value chain selection and innovation prioritization (Section 2.2), an economic analysis assessed implementation costs. A

Microsoft Excel-based CBA template was employed to capture all relevant costs, including initial investments, ongoing implementation, maintenance, and operation for both the BAU scenario and the proposed innovations. Notably, most innovations incurred upfront costs, followed by operation and maintenance expenses. Benefits, however, were primarily realized after the first year of implementation. Future benefits were discounted at a rate reflecting respective country government interest rates, as provided by expert surveys.

For most innovations, the primary benefits stemmed from reduced production costs and improved yields due to enhanced input use precision. Unlike ex-post CBA, which relies on historical data, ex-ante CBA inherently involves uncertainties ([Farrow and von Winterfeldt, 2020](#)). However, in many cases, the anticipated relative yield improvement (coupled with reductions in installation, maintenance, and operational costs) often provides sufficient grounds for estimating benefits associated with specific innovation implementation. Future maintenance and operational costs were considered based on the assumption of performance similar to existing, comparable innovations.

Cost-benefit analysis (CBA) aggregates the present value of all benefits and costs, both private and public, to assess the economic viability of investments. Private benefits and costs accrue directly to those involved in producing and consuming the innovation's associated products. In this study, a farmer-centric ex-ante CBA model was employed to evaluate the profitability of innovations from the perspective of the implementer. This approach focuses on private benefits (e.g., reduced production costs, increased yields) and private costs (e.g., implementation, maintenance) borne by the farmer. Public benefits and costs, also known as externalities (e.g., environmental impacts), are not considered here. Recognizing the time-varying nature of costs and benefits, the analysis incorporates discounting using country-specific prevailing discount rates to account for the time value of money.

2.7 Analytical model and profitability indicators

The benefit associated with innovation is computed as the difference between the net benefits associated with implementing the innovation and the net benefits of conventional or normal farming without any form of improvement also referred to as BAU ([Equation 1](#)).

$$\text{Innovation Net Benefits}_t = \frac{[\sum_{i=1}^n (\text{Innovation Net Benefit}_{jt} - \text{BAU Net Benefits}_{jt})]}{n} \quad (1)$$

Where t stands for the time (in years) that the farmers invest in the innovation j and n is the total number of experts interviewed per specific innovation and its associated BAU. The unit of analysis is standardized to per hectare basis.

This study employs three key profitability indicators: net present value (NPV), internal rate of return (IRR), and payback period (PP). NPV represents the discounted sum of the incremental

net benefits generated by the innovations compared to the BAU scenario over the innovation lifecycle within a specific value chain for each country. A positive NPV and an IRR exceeding the discount rate are generally considered favorable investment indicators. Equation 2 details the NPV calculation.

$$NPV_t^{Innovation} = \left[\sum_{t=1}^T \frac{1}{(1+r)^t} \left(\sum_{i=1}^n \left[P_{nt} \times \Delta Y_{nt}^{Innovation-BAU} - \Delta C_{nt}^{Innovation-BAU} \right] \right) \right] \quad (2)$$

Where T stands for the number of years considered for the NPV calculation, r stands for the discount rate used to calculate the present values of future cash flows, t stands for the time (in years) that the farmers invest in the innovation and n is the total number of key experts interviewed about innovation at a given time, and P stands for price. ΔY and ΔC stands annual change in yield for output and annual change in costs respectively due to the innovation compared to the BAU, respectively.

The discount rate employed reflects the time value of money for farmers, considering the market rate of return on their investments (Howarth, 2009). The IRR, calculated using Equation 3, represents the discount rate at which the NPV of the innovation equals zero (Hartman and Schafrick, 2004). In simpler terms, it is the maximum acceptable borrowing rate for an investment that allows full recovery of costs (installation, maintenance, operation) and achievement of a break-even point (Noori et al., 2018). Innovations with an IRR exceeding the discount rate are generally considered financially viable investments.

$$NPV = \sum_{t=1}^n \left[\frac{B_t - C_t}{(1+r)^t} \right] = 0 \text{ IRR} > 0 \quad (3)$$

Where B_t stands for the accrued benefits at time t , C_t stands for the investment and recurrent costs incurred for innovation at time t , t stands for the period or lifetime of the innovation, and r is the interest rate or discount rate. The payback period (PP) represents the time horizon required for an investment to recover its initial capital outlay. In simpler terms, it reflects the duration needed to recoup the funds invested in installing and maintaining the innovation (Equation 4). PP serves as a simplified metric for assessing the liquidity of an investment, indicating how quickly the investor can regain their initial investment.

$$\text{Payback Period (PP)} = \left[\frac{\text{Investment Cost}}{\text{Net annual Cash Inflows}} \right] \quad (4)$$

2.8 Values used in computing the profitability indicators

To model the physical response curves for activities affected by the innovation, it was assumed that the yields for the products affected by the innovations followed a response function characterized by a lag period, then start increasing and continues to reach maximum and following which a linear plateau is

experienced. The assumption that yields follow a response function characterized by an initial lag period, subsequent increase, and eventual plateau is justified based on several well-documented agricultural phenomena. Firstly, innovations in agricultural practices often require an adaptation period where farmers and systems adjust to new methods, resulting in an initial lag. As the innovation is fully adopted and optimized, yields typically experience a significant increase due to improved efficiencies, better resource utilization, and enhanced crop management practices. Finally, the plateau phase reflects the natural limitations of the innovation, where maximum potential yields are reached, and further increases become minimal, aligning with the diminishing returns principle in agricultural production. This model mirrors empirical evidence observed in numerous agricultural studies, ensuring a realistic and credible representation of yield dynamics over time (see Ng'ang'a et al., 2021 for more details).

2.9 Sensitivity analysis

Uncertainty surrounding key cost and benefit parameters can significantly influence the decision related to economic viability of innovations. To assess the robustness of our findings, this study employed a sensitivity analysis. The initial step involved break-even analysis, which identifies the critical change required in cost or benefit parameters for the Net Present Value (NPV) to reach zero or the initial investment amount for each innovation.

Next, a tornado analysis (SenseIt, 2017) was conducted to visualize the impact of parameter uncertainty on NPV. This analysis began by establishing a baseline scenario with best estimates for all parameters. Subsequently, lower and upper bounds were defined for each uncertain parameter to capture a realistic range of uncertainty. Finally, the NPV was calculated under scenarios where each parameter took on its lowest and highest values, allowing for a visual assessment of the most influential parameters.

Following the tornado analysis, a Monte Carlo simulation using @Risk software (Palisade Corporation, 2013) was performed for a more comprehensive uncertainty analysis. Triangular probability distributions were assigned to each uncertain parameter. Triangular distributions were chosen for their computational efficiency and because they can effectively capture potential tail uncertainties, even though they might exaggerate them to some extent (Thrift and von Winterfeldt, 2021). The base case value served as the most likely value, while lower and upper bounds were selected to encompass a realistic range of uncertainty. By randomly sampling from these parameter distributions, thousands of possible NPV outcomes were simulated ($n=10000$ simulations), generating a distribution of potential net benefits for each innovation.

The results are summarized using the 5th percentile, median, and 95th percentile of the simulated NPV distribution. This approach provides a comprehensive picture of the potential range of net benefits for each innovation, considering the inherent uncertainties in the underlying parameters.

3 Results

This study investigated the impact of three agricultural innovations on crop yield in Lemo, Munesa, and Goba districts. The innovations evaluated were optimal site-specific inorganic fertilizer rate, certified seed, and drainage improvements. Data was collected for a period of 30 years, with yield responses measured from year 1 to year 2 after implementation.

3.1 Yield changes

Table 1 summarizes the average yield per hectare for the BAU, the innovation lifecycle, the time when innovation started to have a physical impact on wheat and when it reached maximum, the innovation lifecycle, the average change in yield per hectare following the implementation of the innovation were estimated from the data collected from the experts. All three innovations resulted in significant yield increases compared to BAU practices. The average yield increase for the optimal fertilizer rate was 677 kg/ha (or 22%), the highest among the three innovations. Certified seeds demonstrated a consistent yield improvement of 603 kg/ha (or 18%), while drainage improvements provided an average yield increase of 617 kg/ha (or 20%).

The data also revealed variability in yield response across districts and practices. The optimal fertilizer rate exhibited the highest variability in both BAU and innovation scenarios, suggesting potential benefits from further tailoring fertilizer application based on local conditions. Certified seeds and drainage improvements showed relatively lower variability, indicating a more consistent response across districts. However, all three innovations result in a positive increase in yield per hectare, demonstrating their effectiveness in improving agricultural productivity. These yield increases (of 18–22%) translate to significant economic benefits for farmers. Increased crop production can lead to higher income, improved food security, and potentially lower food prices for consumers.

3.2 Implementation and maintenance costs

The economic feasibility of each innovation extends beyond yield increases and requires consideration of implementation and

ongoing maintenance costs. The results in **Table 2** reveal a range of costs associated with each innovation. The implementation costs for the optimal fertilizer rate is approximately US\$298.60 per hectare across Lemo, Munesa, and Goba, with an estimated standard deviation of US\$74.09. Year-one maintenance costs an average of US\$235.00 per hectare. In Munesa and Goba, certified seed implementation averages US\$235.30 per hectare, with a standard deviation of US\$58.83. However, year-one maintenance costs for certified seeds are higher at US\$331.00 per hectare. Drainage improvements, implemented only in Munesa, have a higher average implementation cost of US\$302.40 per hectare with a standard deviation of US\$75.60. Year-one maintenance costs for drainage are US\$243.90 per hectare. The high maintenance costs can be attributed to several factors. Firstly, the region's specific geographic and hydrological conditions may require more extensive and frequent maintenance efforts to ensure effective drainage. Studies have shown that areas with higher rainfall variability and poor soil drainage capacity necessitate significant and ongoing investments in drainage infrastructure to prevent waterlogging and maintain soil health (Awulachew, 2006).

3.3 Financial returns

The results reveal that all three agroecological innovations yield positive NPVs, indicating strong long-term profitability for farmers (**Table 3**). Among them, certified seed option emerges as the most lucrative, with the highest NPV of US\$2,531, followed closely by the optimal fertilizer rate at US\$2,371, and drainage at US\$2,099. In addition, given the prevailing market discount rate of 10%, both the certified seed and optimal fertilizer rate demonstrate remarkably high IRRs each exceeding 100%. The drainage option also performs well, with an IRR of 106%. Notably the payback period for all three innovations is just one year, underscoring their capacity to quickly recover the initial investment.

These results, characterized by high NPVs and IRRs far above the market discount rate, suggest that each of these innovations presents a financially attractive opportunity. Investing in any of the three would likely lead to substantial financial gains. However, the certified seed option stands out as the most financially appealing, given its superior NPV and IRR, making it the best investment choice in terms of potential returns.

TABLE 1 Average yield impact of agricultural innovations in Ethiopia.

Districts covered	Innovation Name	Evaluation period (Years)	Response start (Year)	Response reach maximum (Years)	Average yield BAU (kg/ha)	Average yield (Innovation) (kg/ha)	Average increase (Kg/ha)
Lemo, Munesa, Goba	Optimal site-specific inorganic fertilizer rate*	30	1	2	3045 ± 979	3722 ± 1204	677
Munesa, Goba	Certified seed*	30	1	2	3344 ± 909	3947 ± 1060	603
Munesa	Drainage**	30	1	2	3033 ± 776	3650 ± 900	617

**, *, stand for n = 2 and n = 6 respectively; evaluation period is synonymous with innovation lifecycle.

TABLE 2 The cost of implementation and maintenance and operation of each innovation.

Districts covered	Innovation Name	Implementation (US\$/ha)	Maintenance and operation (US\$/ha/Year)
Lemo, Munesa, Goba	Optimal site-specific inorganic fertilizer rate*	298.6 ± 74.09	235 ± 21.36
Munesa, Goba	Certified seed*	235.3 ± 7.56	331 ± 22.50
Munesa	Drainage**	302.4 ± 74.09	243.9 ± 14.02

**, *, stand for n = 2 and n = 6 respectively.

Supplementary Tables A1–A3 provide detailed cash flow statements in real values for the total investments in “Optimal site-specific inorganic fertilizer rate,” “Certified seed,” and drainage innovations, respectively. These tables further illustrate the financial differences between the “with” and “without” scenarios for each innovation.

3.4 Sensitivity results

3.4.1 “Optimal site-specific inorganic fertilizer rate” innovation

The sensitivity analysis for the “optimal site-specific inorganic fertilizer rate” innovation (Figure 1) offer a novel probabilistic insight into its financial viability highlighting the renage and likelihood of potential outcomes. Using 10,000 Monte Carlo simulations, the analysis predict with 90% certainty that the NPV will range between \$1,117 and \$4,341, providing wheat farmers with a nuanced understanding of the financial risks and rewards. The mean NPV of \$2,597 reinforces the positive expected value, signaling a promising return on investment.

What sets this analysis apart is its ability to account for uncertainty, a key factor often overlooked in traditional evaluations of agricultural innovations. By integrating probabilistic methods, the study moves beyond static evaluations, offering farmers and stakeholders a clearer, data-driven picture of potential financial outcomes. Notably, the analysis reveals a very low probability of negative returns, further strengthening the case for adopting this innovation under varying market and environmental conditions.

The profitability of the “optimal site-specific inorganic fertilizer rate” innovation is influenced by several key factors. Sensitivity analysis (Figures 2, 3) indicates that annual changes in wheat yield have the greatest impact on NPV, accounting for 66% of the

variation. Additionally, the market price per kilogram of wheat and the discount rate play significant roles, contributing 12% and 10% to NPV variation, respectively, while total operation costs account for 8%. These results highlight the need to consider not only direct input costs, such as labor, but also external factors like market fluctuations and long-term financial planning. Understanding how these variables interact is essential for evaluating the potential benefits for wheat farmers in Ethiopia.

3.4.2 “Certified seeds” innovation

The sensitivity analysis results (Figure 4) provides a probabilistic view of the potential net present values (NPVs) for the “certified seeds” innovation. The analysis indicates a 90% probability that the NPV will range between \$557 and \$3,412, based on 10,000 simulations. With a mean NPV of \$1,870, the innovation shows a strong positive expected value. Overall, these results are highly encouraging for wheat farmers, as they suggest a very low risk of negative return from this investment.

The profitability of the “Certified seeds” innovation is influenced by several key factors. The analysis (Figures 5, 6) shows that annual changes in wheat yield have the most significant impact, accounting for 59% of the variation in NPV. Additionally, the market price per kilogram of wheat and labor costs play important role, contributing 17% and 11% to NPV variation, respectively. The prevailing discount rate, which account for the time value of money, influences NPV by 6%. These findings highlights the need to consider not only direct input costs, such as labor, but also external factors like market fluctuations when evaluating the potential benefits of this innovation’s potential benefits for wheat farmers in Ethiopia.

4 Discussion

This study investigated the economic viability of three agroecological innovations (optimal fertilizer rate, certified seed, drainage) for farmers in Lemo, Munesa, and Goba districts. The findings hold significant implications for promoting inclusive growth in the rural communities of Ethiopia.

All three innovations: optimal fertilizer rate, certified seed, and drainage, demonstrated substantial yield increases compared to traditional practices. Increased production can contribute to improved food security at the household level and potentially contribute to lower food prices for consumers. This aligns with the concept of inclusive growth, which emphasizes not just

TABLE 3 The change in NPV associated with the innovations at the prevailing discount rates.

Innovation Name	NPV (US\$)	IRR	Payback period
Optimal site-specific inorganic fertilizer rate*	2,371	106%	1
Certified seed*	2,531	117%	1
Drainage**	2,099	106%	1

**, *, stand for n = 2 and n = 6 respectively, Market discount rate.

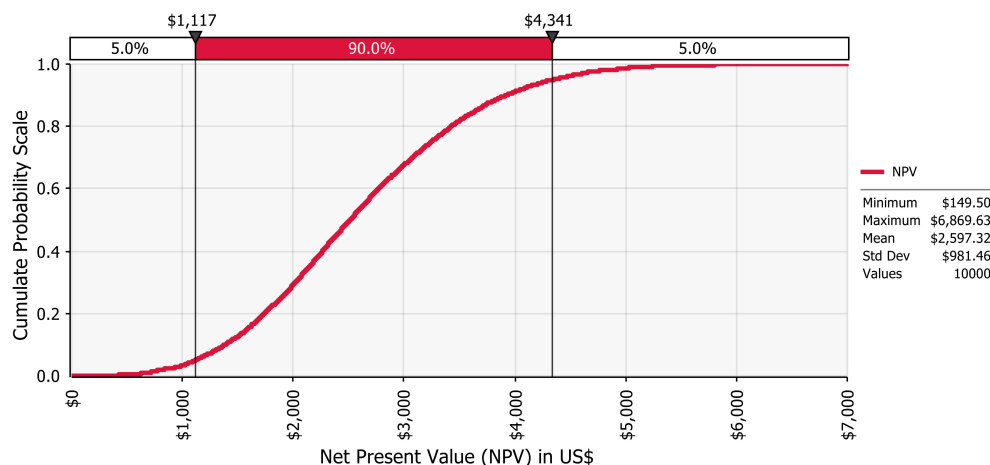


FIGURE 1

The cumulative probability distribution of Net Present Value (US\$/ha) for "Optimal site-specific inorganic fertilizer rate" innovation in Ethiopia.

economic prosperity but also equitable distribution of benefits. The finding that certified seeds can increase wheat yields in Ethiopia by 18% is significant compared to the results observed in other countries. Such as Pakistan (15% yield increase), India (10% yield increase), the United States (5% yield increase), and Australia (8% yield increase) (citations), Ethiopia's potential for yield improvement through certified seeds appears considerably higher. This suggests that Ethiopian wheat varieties may be particularly responsive to the genetic improvements found in certified local seeds. Several factors could explain this higher potential. Ethiopia's traditional wheat varieties might be particularly susceptible to diseases or pests that certified seeds offer resistance to (citations). Additionally, the climate and soil conditions in Ethiopia might be more conducive to the improved performance of certified varieties.

A 22% yield increase due to optimal fertilizer rate intervention translates to a significant boost in wheat production. This can have positive economic implications for Ethiopian farmers, leading to increased incomes and improved livelihoods. Furthermore, it can contribute to national food security by increasing domestic wheat

production and potentially reducing dependence on imports (Anteneh and Asrat, 2020).

The projected rise in agricultural output due to these innovations has the potential to create a ripple effect through the Ethiopian rural economy. Increased yields can translate to a demand for more labor across various parts of the agricultural value chain. This could include tasks like planting, weeding, harvesting, and post-harvest processing. Wider adoption of these innovations could contribute to addressing this need by generating additional employment opportunities, potentially improving livelihoods and reducing rural-urban migration (Jayne and Sanchez, 2021).

Furthermore, ensuring equitable access to these innovations can be instrumental in empowering women farmers who play a crucial role in Ethiopian agriculture. Research suggests that women often face challenges in accessing resources and training opportunities (Williams et al., 2022). By facilitating women's participation in trainings on these innovations and ensuring their access to credit and resources, policymakers can create a more inclusive environment. This can lead to increased agricultural productivity

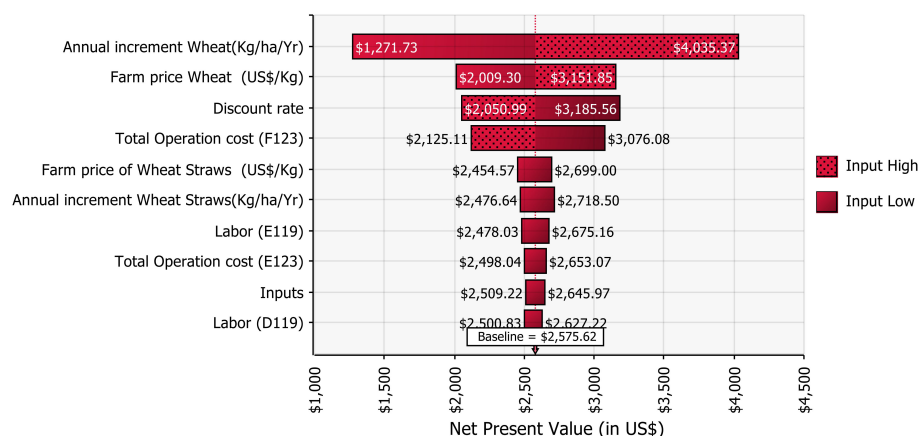


FIGURE 2

Inputs ranked by effect on the mean Net Present Value for "Optimal site-specific inorganic fertilizer rate" innovation in Ethiopia.

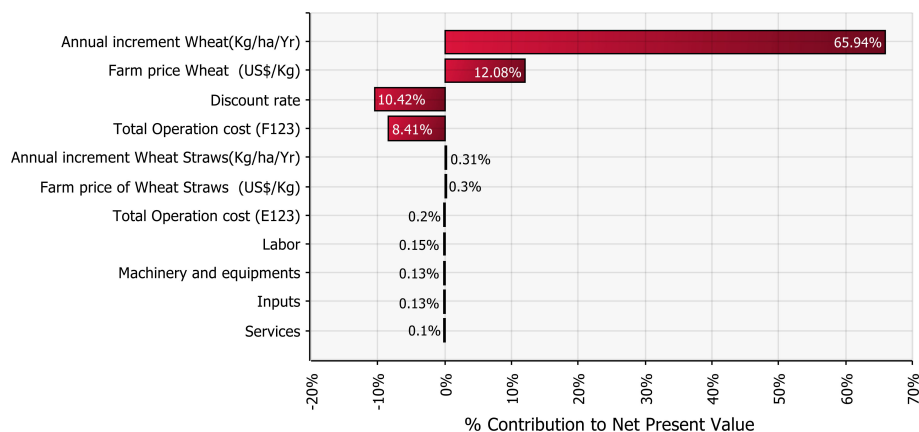


FIGURE 3

Percentage contribution to variation in Net Present Value for "Optimal site-specific inorganic fertilizer rate" innovation in Ethiopia.

managed by women, contributing to household income and overall well-being within communities. Increased agricultural productivity has been shown to have positive correlation with and rural poverty reduction (World Bank, 2009).

The study employed economic measures (NPV, IRR, payback period) to assess the long-term profitability of each innovation. Notably, all three options emerged as financially attractive, with certified seed demonstrating the highest NPV and IRR. The IRR for both certified seed and optimal fertilizer rate exceeded 100%, significantly higher than the prevailing discount rate of 10%. This suggests that these innovations offer a very high potential return on investment, exceeding the opportunity cost of capital. In simpler terms, the return on investment for these practices is projected to be much higher than the interest rate farmers might pay to borrow money to implement them.

While this study highlights the high potential return on investment (IRR) for certified seeds in Ethiopia, it contrasts with findings elsewhere (where)? that show negative returns for wheat production (citations). This discrepancy could be due to several factors. The positive IRR in our study suggests that certified seeds

can significantly increase yields and profitability (Elias et al., 2017). Conversely, the negative ROI could be attributed to the use of low-quality improved seeds and wheat leaf rust, factors that can be mitigated through access to high-quality certified seeds and proper disease management practices.

This study underscores the economic viability of all three innovations (optimal fertilizer rate, certified seed, drainage) not just through their high potential returns, but also their short payback periods. A short payback period signifies that farmers can recover their initial investment within a single harvest season. This aspect, combined with the high returns on investment (NPV and IRR) discussed earlier, presents a powerful incentive for wider adoption, particularly among resource-constrained smallholder farmers.

A short payback period translates to reduced financial risk for farmers adopting these innovations (Akinji et al., 2022). Knowing they can recoup their investment quickly can incentivize them to experiment with these practices and potentially see the benefits firsthand. This can lead to a snowball effect, where initial success stories encourage other farmers to adopt the innovations, accelerating the diffusion of these technologies. Furthermore, the

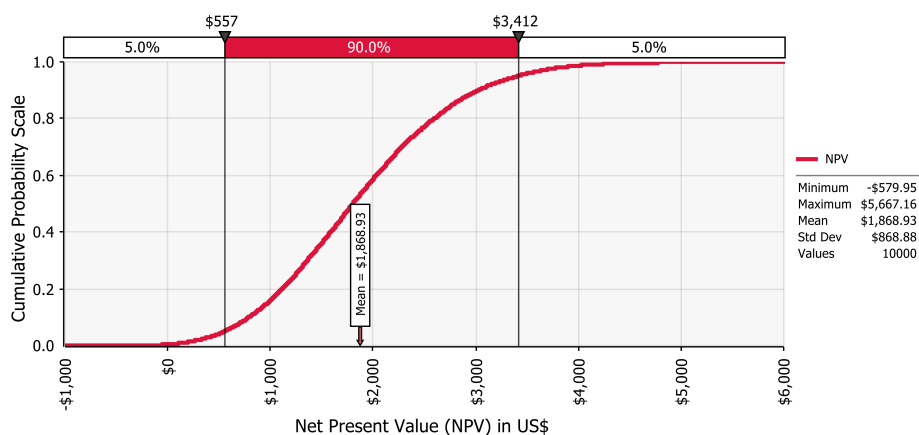


FIGURE 4

The cumulative probability distribution of Net Present Value (US\$/ha) for "Certified seeds" innovation in Ethiopia.

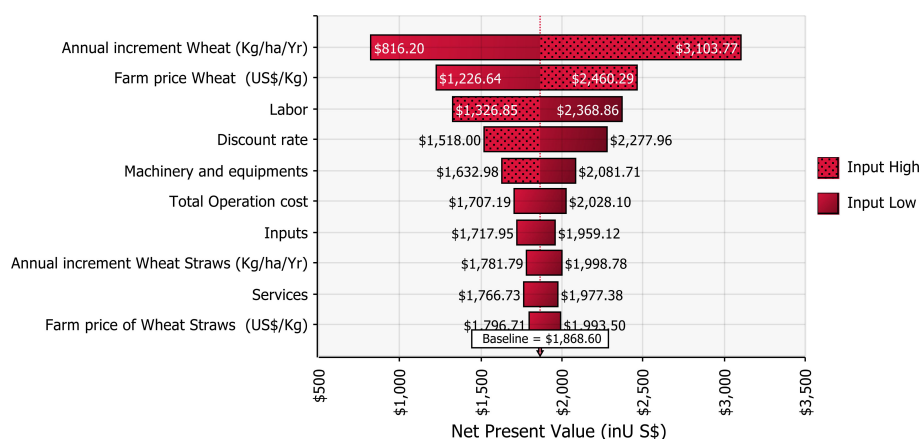


FIGURE 5

Inputs ranked by effect on the mean Net Present Value for “certified seeds” innovation in Ethiopia.

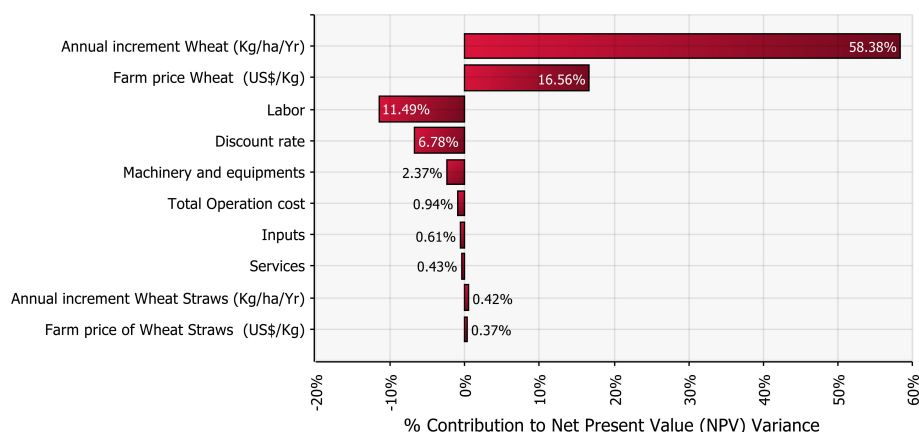


FIGURE 6

Percentage contribution to variation in Net Present Value for “Certified seeds” innovation in Ethiopia.

positive cash flow generated within a year can improve household food security and empower farmers to invest in other farm improvements, creating a cycle of continuous progress.

The optimal choice for individual farmers will still depend on factors like risk tolerance, crop type, and market conditions. This highlights the need for targeted extension services. Extension efforts should emphasize the rapid return on investment associated with these innovations and tailor recommendations based on individual circumstances. Financial inclusion initiatives like micro-loans or input credit programs specifically designed with the payback periods in mind can make these innovations more accessible to smallholder farmers.

By focusing on the combined strengths of short payback periods, high potential returns, and targeted support mechanisms, policymakers can create a compelling case for wider adoption and attracting private sector investments and impact investors. This can unlock the transformative potential of these agricultural innovations for boosting productivity, improving livelihoods, and fostering inclusive growth in rural communities.

While the economic benefits are promising, long-term sustainability and synergies of combining several practices requires further investigation. The potential impact of these practices on soil health and environmental factors needs to be assessed. Research by [Abhijeet et al. \(2023\)](#) emphasizes the importance of integrating sustainability considerations into agricultural development strategies. The successful adoption and diffusion of these innovations relies heavily on effective knowledge dissemination and capacity building for farmers. Collaboration with extension services, farmer associations and multi-stakeholder platforms is crucial to ensure farmers understand the benefits, implementation requirements, and potential risks associated with each innovation.

Despite the high potential returns on investment evidenced by the IRR, a crucial question arises: why are these innovations not being adopted at scale by farmers? Research suggests several reasons for this paradox. Limited access to information and knowledge about the innovations, coupled with risk aversion among farmers, can be significant barriers. Additionally, even with high potential returns,

upfront costs (i.e., US\$ 329, US\$325 and US\$322 per hectare for “Optimal fertilizer”, “certified seeds” and drainage innovations respectively; [Supplementary Tables A1–A3](#)) and lack of access to credit, particularly for smallholder farmers, can hinder adoption.

Another key factor hindering wider adoption is the unavailability of quality seeds at the right place and time ([Abebe et al., 2023](#)). Insufficient certified seed production and distribution networks can leave farmers without access to these improved varieties when they need them most for planting ([Beshir, 2013](#)). This is compounded by a poor promotion system. Limited awareness about the benefits of certified seeds and inadequate information on their proper use can leave farmers hesitant to adopt them.

This situation highlights the need for a two-pronged approach. Firstly, investing in the seed production and distribution system is crucial to ensure a reliable supply of certified seeds throughout the planting season and across all regions. Secondly, strengthening seed promotion efforts through extension services and farmer training programs can raise awareness about the advantages of certified seeds and equip farmers with the knowledge required to utilize them effectively. By addressing these challenges, policymakers can bridge the gap between the potential and reality of certified seed adoption, unlocking their power to contribute to agricultural productivity and food security in Ethiopia.

To bridge this gap and ensure the scaling up of these practices, several policies and institutional responses are necessary. Governments, policymakers and private sector stakeholders can play a critical role by:

- Strengthening extension services: Investing in extension services to bridge the knowledge gap and provide farmers with training and information on these innovations.
- Facilitating access to credit: Developing financial inclusion initiatives such as micro-credit programs or loan guarantees to help farmers overcome upfront costs.
- Risk mitigation strategies to de-risk food systems: Exploring crop insurance schemes or other risk mitigation strategies to incentivize adoption, particularly for risk-averse farmers.
- Market access and infrastructure development: Improving market access for farmers to ensure they can reap the benefits of increased production through better prices.

The sensitivity findings underscore the critical role of yield fluctuations and market conditions in determining the financial success of these agronomic practices. A study by [Feuerbacher et al. \(2018\)](#) recognized a discernible correlation between socio-economic status and the accessibility of markets, underscoring the importance of affordability in agricultural practices and ease of sale.

5 Conclusion

This study underscores the significant economic viability of three agroecological innovations—optimal fertilizer rate, certified

seed, and drainage improvements—within the Lemo, Munesa, and Goba districts of Ethiopia. The substantial yield increases observed from these innovations can significantly enhance household food security and contribute to lower food prices, aligning with the principles of sustainable growth.

The 22% yield increase from optimal fertilizer rates and the high return on investment (exceeding 100% IRR) for certified seeds and fertilizers highlight their economic benefits. The short payback periods associated with these innovations reduce financial risks and provide strong incentives for adoption among smallholder farmers. These innovations not only boost productivity and income but also create employment opportunities, thereby fostering rural economic growth.

Equitable access to these innovations is essential, particularly for women farmers who face significant barriers in accessing resources and training. Empowering women through targeted training and access to credit can enhance agricultural productivity and contribute to community well-being. The adoption of these innovation among the youth and women can be boosted through strengthened extension services, improved seed production and distribution, financial inclusion initiatives. By implementing these measures, policymakers and stakeholders can unlock the transformative potential of agroecological innovations, driving productivity, improving livelihoods, and fostering inclusive growth in Ethiopia's rural. In the future, further research on the long-term sustainability and environmental impact of these practices is necessary to ensure their sustainable adoption and scaling up.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by International Center for Tropical Research (CIAT) Institutional Review Board (IRB). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

SN: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. SO: Data curation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. DT: Data curation, Investigation, Writing – review & editing. DA: Writing – original draft. JM: Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing.

Funding

The author(s) declare that financial support was received for the research, authorship, and/or publication of this article. The Agroecological Transitions for Building Resilient and Inclusive Agricultural and Food Systems (TRANSITIONS) programme is funded by the European Commission through its DeSIRA initiative and managed by the International Fund for Agricultural Development (IFAD). This publication was produced by the “Private Sector Incentives and Investments (PSII) for Climate Change, Resilience and Environmental Sustainability” project under the European Commission - IFAD Grant Number: 2000003771 and CGIAR initiative on Transformative Agroecology.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary Material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fagro.2024.1502786/full#supplementary-material>

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RECEIVED 25 November 2024

ACCEPTED 15 January 2025

PUBLISHED 07 February 2025

CITATION

Altieri MA, Nicholls CI, Montalba R, Vieli L and Vazquez LL (2025) Agroecology and the limits to resilience: extending the adaptation capacity of agroecosystems to drought. *Front. Agron.* 7:1534370. doi: 10.3389/fagro.2025.1534370

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Agroecology and the limits to resilience: extending the adaptation capacity of agroecosystems to drought

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Given the unpredictability, increasing frequency and severity of climatic events, it is crucial to determine the adaptation limits of agroecological strategies adopted by farmers in a range of environments. In times of drought many smallholders' farmers cope with stress using a series of crop diversification and soil management strategies. Intercropping and agroforestry systems complemented with mulching and copious organic matter applications can increase water storage, enhancing crops' water use efficiency. Although an overwhelming number of studies demonstrate that these agroecological designs and practices are associated with greater farm-level resilience, it is important to recognize the limits of resilience. The aim of this paper is to assess the limitations of agroecological practices in enhancing the ability of agroecosystems to adapt to climate change under extended drought stress which may overwhelm crops' adaptation response. A set of agroecological practices that can extend such limits under prolonged water stress scenarios are described. Two methodologies to assess farms' resilience to drought provide useful tools, as they can assist farmers and researchers in identifying the practices and underlying mechanisms that reduce vulnerability and enhance response capacity allowing certain farm systems to better resist and/or recover from droughts. Clearly, reducing farmers exposure to drought requires collective actions beyond the farm scale (i.e. restoring local watersheds to optimize local hydrological cycles) aspects not explored herein. When climatic events are compounded by uncertainties imposed by external economic and political conditions, farmers' abilities to overcome adversity may be reduced, emphasizing the importance of policy support, a dimension beyond the scope of this review.

KEYWORDS

drought, limits of resilience, agroecology, adaptation, mulching, soil organic matter, diversification

1 Introduction

Earlier than predicted by the scientific community, the world is already facing a series of extreme climatic events (droughts, hurricanes, floods, heat-waves, sea level rise, etc.) that threaten agricultural production and food security in many regions of the world. Modern agricultural systems characterized by monocultures linked to pesticides and transgenic crops are not shifting in ways that will protect such simplified systems from current and expected shifts in climate change. Rather, specialization and intensification pressures driven by short-term economic benefit, force farmers towards specialization and intensification at significant risk to long-term agricultural stability (D'Agostino and Schlenker, 2016). On the other hand, droughts, storms and floods pose a significant threat to more than 475 million smallholder farmers who despite in producing 50–70% of the world's food are very vulnerable to climate change as most live in fragile landscapes (hillsides, flood plains, etc.) and who have few assets to fall back and limited ability to recover from intense climatic events (Lobell and Gourdji, 2012). As long as these socio-economic trends hold into the future, maintaining crop productivity in large and small farms in the face of anticipated climatic events will be a major challenge.

Emerging evidence suggests that increasing the diversification of agricultural systems at the field and landscape level, and enhancing soil organic matter and biological activity, are key strategies to improve the resilience of agricultural systems to climate variability (Altieri et al., 2015). Although the overwhelming majority of studies demonstrate that agroecological designs and practices are associated with greater farm-level resilience protecting farmers against climatic extremes, it is important to recognize the limits of resilience. The ability of agroecosystems to adapt to climate change has limits delineated by capacity thresholds, after which climate damages begin to overwhelm the adaptation response. Even with scaled-up adaptation strategies, the limits of adaptation can often be reached under prolonged and severe climatic stress (Kragt et al., 2013).

Given the unpredictability, increasing frequency and severity of climatic events, it is crucial to determine the adaptation limits of agroecological strategies adopted by farmers in a range of environments. A strong hurricane or prolonged drought could lead to farming system degeneration and failure. The adaptation limit threshold for each farm, the pathways of degradation or failure, and whether the climate impacts suffered represent temporary (recoverable) or permanent losses, will depend on the agroecological features of each farm such as levels of crop diversity, genetic diversity, landscape matrix, soil organic matter, as well as farmers responsive capacity (Córdoba et al., 2019).

Building on what is already known about the degree to which farmers can adapt to a changing climate, the goal of this article is to try to understand and define where and when limits to adaptation to drought can be reached in a particular agro-landscape. Many adaptation measures have been suggested to reduce the vulnerability of farmers to prolonged droughts, but the extent to which those can be efficiently extend and/or postpone threshold limits under severe and prolonged water stress is not known.

2 Impacts of droughts

Industrial agriculture which occupies about 70–80% of the global agricultural surface, is part of the problem by emitting no less than 30% of greenhouse gases. On the other hand, large-scale monocultures which dangerously reduce crop genetic and species diversity, exhibiting a high level of ecological homogeneity, makes them particularly vulnerable to climate change (NRC, 1972). In the late twentieth century in the USA, 60–70% of the total bean area was planted with 2–3 bean varieties, 72% of the potato area with four varieties, and 53% of the cotton area planted with three varieties, demonstrating how modern agriculture is shockingly dependent on a handful of varieties for its major crops (Robinson and Wallace, 1996). This fragile ecological status of industrial agriculture represents a major threat to humanity's food security.

The estimated global yield loss each year due to drought is estimated at around USD 10 billion. Severe droughts cause substantial decline in crop production leading to 21 and 40% yield reductions in wheat and maize when grown in monocultures, which is the norm (Daryanto et al., 2017). Vulnerability to droughts was evidenced in the United States in 2012, when the worst drought in 50 years occurred, severely affecting crop production in 26 of the 52 states and covering at least 55% of the U.S. land area. In the US Midwest, specialization in rain-fed maize and soybean production, makes this region increasingly sensitive to drought, leading in 2012 to reduced maize yields by ~25% (Boyer et al., 2013).

After four years of drought in California (2011–2015), large areas of land (more than 250,000 hectares) were removed from cultivation due to lack of water, representing losses of US\$1.8 billion and a reduction of 8,550 jobs. In 2014, harvested acreage was 6.9 million acres lower than at any time in the past 15 years and crop revenue declined by US \$480 million (Cooley et al., 2015).

On the other hand, resource poor farmers living in vulnerable landscapes are particularly sensitive to climate change. Recent studies suggest that by 2025 climate stress may reduce bean production in Central America by more than 20% and maize yields by as much as 15%. In Honduras, the predicted production losses could amount to about 120,000 t annually, valued at about US\$40 million (Eitzinger et al., 2012). The 2014–2016 drought in the dry Pacific region of Central America resulted in 1.6 million people becoming food insecure and 3.5 million in need of humanitarian assistance. The projected mean precipitation decrease will be accompanied by more frequent dry extremes in all seasons, leading to grain yield reductions in Mexico up to 30% by 2080 (Donatti et al., 2019). The most climatically vulnerable are small-holders who farm on steep lands with thin soils, depending on rainfed agriculture while lacking technical and/or financial support. In addition, poor rural households have difficulty coping with climate change where infrastructure (equipment and roads) is inadequate, access to natural resources (water and land) is limited and social capital and government support is weak.

3 Efforts to build resilience

Despite the serious effects of climate change on small-scale agriculture, data from model predictions often ignore the adaptive capacity of small farmers who use several agroecological strategies and socially mediated solidarity networks to cope with and even prepare for extreme climatic variability. Many researchers have found that despite their high-exposure sensitivity, indigenous people and local farming communities are actively responding to changing climatic conditions and have demonstrated their resourcefulness and resilience in the face of climate change (Morton, 2007).

Strategies such as maintaining crop genetic and species diversity in fields and herds provide a low-risk buffer in uncertain environments (Gil et al., 2017). A review of 172 case studies and project reports from around the world shows that agricultural biodiversity contributes to resilience through a number of strategies that are often combined: the protection and restoration of watersheds, the sustainable use of soil and water resources, agroforestry, diversification of farming systems, various adjustments in cultivation practices and the use of stress-tolerant crops (Mijatovic et al., 2013).

4 Adapting to droughts

Most farmers efforts to cope with drought are usually directed at minimizing risk. Scaling back on production which involves a reduction in the size of the cultivated area, by as much as 25%, or establishing “protected” community gardens are common adaptive responses after a drought. In times of drought many smallholder farmers cope with stress planting more root and tuber crops, increasing consumption of fruits to replace lost basic grains, selling fire wood and animals as an alternative income source, reducing food consumption, selling crops for lower prices, and seeking help from governments and other organizations (Harvey et al., 2018).

A common strategy is resorting to wild food harvest such as weeds that in Meso America, traditional farmers usually call “quelites or arvenses”, important sources of vitamins, minerals and protein (content of edible wild plants can usually range from 1.3% to 7.5% of freshweight) thus improving the nutritional quality of local diets (Ebel et al., 2024). In Tlaxcala Mexico a typical milpa system may produce up to 13.2 tons of quelites, each family consuming 3 kg 2-3 times/week. This is important in time of crop failure due to drought, where certain weed species of the genus *Portulaca*, *Amaranthus* and *Chenopodium* are more tolerant than maize and beans to water stress (Altieri and Trujillo, 1987).

In dry environments, farmers who are fortunate to experience a small level of rainfall and are able to harvest some water from roofs and catchment areas, an option is to establish small areas with new, off-season vegetables using the limited collected water. Drought adaptation measures also include choosing sturdier varieties and shifting to other crops entirely, to adopting/improving irrigation systems. In sub-Saharan Africa much emphasis has been given to promoting ancient crops which exhibit drought tolerance such as teff, fonio, various millet varieties, sorghum, cassava and several legumes species such as pigeon peas and cowpeas.

Measures directed at breaking vulnerable monocultures imply a redesign of the farming system which includes adoption of soil management practices such as using a thick layer of mulch and copious applications of compost, to diversification practices such as intercropping and agroforestry systems. Natarajan and Willey (1986) examined the effect of drought on enhanced yields with polycultures by manipulating water stress on intercrops of sorghum and peanut, millet and peanut, and sorghum and millet. All the intercrops over-yielded consistently at five levels of moisture availability, ranging from 297 to 584 mm of water applied over the cropping season. Quite interestingly, the rate of over-yielding actually increased with water stress, such that the relative differences in productivity between monocultures and polycultures became more accentuated as stress increased.

Many intercropping systems also improve the water use efficiency compared to monocultures. Water-utilization efficiency by intercrops usually exceeds that of sole crops, often by more than 18% and sometimes by as much as 99%. They do so by promoting the full use of soil water by plant roots, increasing the water storage in the root zone, and reducing inter-row evaporation, but also by controlling excessive transpiration and creating a special microclimate advantageous to plant growth and development (Lithourgidis et al., 2011).

Higher resistance to drought may be more common in cropping systems that exhibit higher levels of soil organic matter content, which in turn enhances the soil's moisture holding capacity, leading to higher available water for plants, which positively influences resistance and resilience of crop plants to drought conditions. Hudson (1994) showed that as soil organic matter content increased from 0.5% to 3%, available water capacity more than doubled. Mulching is central to farmers' adaptation to dry conditions which helps conserve soil moisture by reducing evaporation, thereby more moisture is accessible near the plant roots, extending the time for plants to absorb water (Sharma and Bhardwaj, 2017).

Agroforestry systems buffer crops from large fluctuations in temperature (Lin, 2011), thereby keeping the crop closer to its optimum conditions. Shaded coffee systems have shown to protect crops from decreasing precipitation and reduced soil water availability because the over story tree cover is able to reduce soil evaporation and increase soil water infiltration (Lin, 2007).

Larger scale farmers may adapt to stressful growing conditions by adopting diversified rotations. A recent study showed that a 7% higher maize yield during hot and dry years in a diversified five-crop rotation than in a simpler maize-soybean rotation. Such gains resulted from improved soil properties, such as increases in soil water capture and storage and abundance of beneficial soil microbes (Renwick et al., 2021). More diverse rotations also showed positive effects on yield under unfavorable conditions, by reducing yield losses from 14.0%–89.9% in drought years. Analysis of 11 long-term experiments comprising 347 site-years and ~11,000 observations across the US and Canada showed that crop-rotational diversity can reduce the risk of low maize yields during droughts (Bowles et al., 2020). Another strategy commonly used by commercial farmers is the use of cover crop mixes planted before the main grain crop. A mix of rye, hairy vetch, crimson clover planted before corn, exhibited 20 mm greater soil water storage compared to no cover

crop before corn. Estimated evapotranspiration was lower for systems with cover crop mix, exhibiting also greater estimated infiltration rates (Schomberg et al., 2023).

Farmers can rely on three strategies against drought stress: plant escape, avoidance and tolerance, involving mechanisms that range from early crop flowering to increase of water uptake from well-established root systems (Fahad et al., 2017). Figure 1, lists the most effective agroecological strategies with potential to enhance such mechanisms.

5 Methodologies to assess resilience of agroecosystems to drought

Resilience is defined as the ability of an agroecosystem to absorb disturbances while retaining its organizational structure and productivity due to its ability to adapt to stress and change following a perturbation (Cabell and Oelofse, 2012). Thus, a “resilient” agroecosystem would be capable of providing food production, when challenged by a severe drought. Researchers have developed methodologies aimed at assessing the resilience of agroecosystems by estimating its vulnerability (refers to the degree to which an agroecosystem is susceptible to the impacts of drought) and the response capacity (ability of both farmers and their farming systems to mitigate, resist and recover from threat like drought). Vulnerability decreases resilience while higher response capacity enhances it, therefore farms exhibiting low vulnerability and high response capacity values are considered more resilient (Altieri et al., 2015). Two of such methodologies are presented below.

5.1 Cuban case study

A study of the perception of farmers and local technicians on sensitivity to drought, was carried out on three integral farms (livestock-agriculture-forestry) undergoing agroecological transition, located in suburban areas of the province of Havana, Cuba: “La Victoria” (24.48 hectares, Marianao municipality),

“Media Luna” (6.5 hectares, Habana del Este municipality), “La China” (7.10 hectares, La Lisa municipality) (Vázquez et al., 2015).

To determine the resilience of farms to drought, the resilience capacity (RCd) provided by specific agroecological designs and management practices was contrasted with the sensitivity to drought expressed by natural resources (SNRd) (Vázquez et al., 2016). The drought resilience capacity (RCd) was determined using the following indicators:

5.1.1 Resistance-absorption

Ability of the agroecosystem to resist-absorb the physical and prolonged effects of drought, which was determined by indicators such as: complexity of the landscape matrix, complexity of the production system, composition of agrobiodiversity, level of soil cover, soil management practices, water access, and design of cropping and livestock systems.

5.1.2 Recovery

Ability of the agroecosystem to return to the productive state prior to the incidence of the event, calculated using state of the productive infrastructure, availability of means of production, capacities of the support infrastructure, reduction of external energy, capacity for self-sufficiency in food and labor, capacity for food self-sufficiency for working animals, capacity for integrating bioinputs for crop nutrition and health of crops and animals, as indicators.

5.1.3 Transformability

Capacity of the production system to achieve resilience capabilities influenced by public policies and the adaptability capabilities and skills of farmers. It is assessed through the following indicators: level of education of workers, gender and generational equity, capacity for self-organization, benefits for workers, participation in reciprocal exchanges, behavioral perception of the principles of agroecology, participation in innovations, capacity for management of financing, level of productive stability, level of biosafety, access to agricultural extension services. RCd values above 0.50 indicate that the

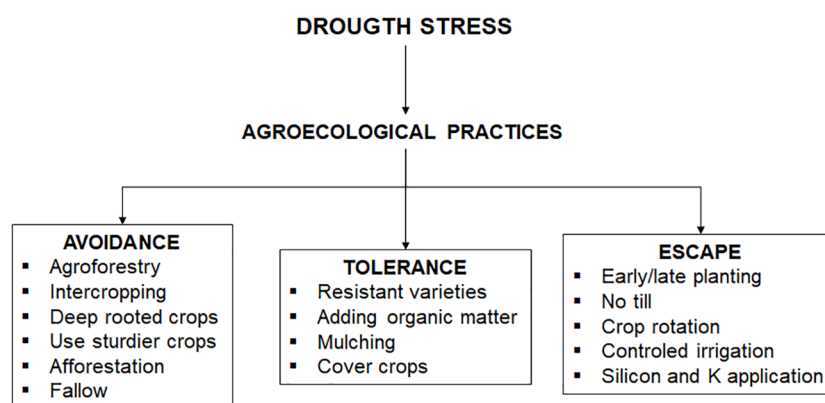


FIGURE 1

Agroecological practices commonly used to implement the strategies of avoidance, tolerance and escape from droughts.

production system is starting to exhibit drought resilience capabilities; values around 1.0 denote advancement towards a state of resilience and values above 1.5 evidence high resilience capabilities.

5.1.4 The sensitivity of natural resources

The sensitivity of natural resources (SNRd) was determined through two components and their respective indicators: exposure to the event (drought frequency and duration) and sensitivity of crops, animals, soil and water supply. SNRd was considered very high when the value obtained was above 0.8; high for values between 0.6–0.8; medium when values ranged between 0.4–0.59; low when values 0.2–0.39 and very low below with values < 0.2.

The three farms exhibited similar resistance-absorption values (between 0.59 and 0.72) and was limited mainly by the low structure of the production system matrix and poor spatial/temporal design of crop and livestock systems. “La Victoria” farm (0.27) and “Media Luna” (0.42) showed low recovery values due to lower availability of means of production, lower infrastructure and low food self-sufficiency for people and animals. “La China” (0.72) exhibited higher recovery values due to greater infrastructure, access to inputs and food self-sufficiency. Transformation ability was greater for the “La China” farm (0.79), followed by “Media Luna” (0.61) and “La Victoria” (0.51). The variables that most limited transformability were: lack of self-organization and finance management, low productive stability and access to extension services.

The General Resilience Index to droughts (GRId) was determined with the following equation: $GRId = RCd / SNRd$ (Vázquez et al., 2019). In summary the lowest drought resilience capacity was exhibited by farm La Victoria (GRId=0.66). The GRId for Media Luna was 0.93 (medium) and La China exhibited a high GRId value 3.21 reflecting high resilience capacities (values above 1.5). The three farms are above the drought resilience

threshold ($GRId > 0.5$), evidencing that production systems under agroecological transition acquire resilience. In the three studied farms, the drought resilience capacity (RCd) is inverse to the sensitivity of natural resources (SNRd) of productive importance such as crops, animals, soil, water supply. Clearly results indicate that as resilience capabilities increase, sensitivity decreases (Figure 2).

5.2 Chilean case study

In the Araucania region of Chile, socio-ecological resilience was evaluated in 177 peasant farming systems differentiated by the cultural ethnicity of the farmers: Mapuche, Chilean and descendants of European settlers, located in an area where droughts are increasing in frequency and intensity due to climate change (Montalba et al., 2015).

Using a series of indicators defined in a participatory manner, farm resilience was estimated based on vulnerability of farms to drought and on the response capacity of farmers. Vulnerability indicators included (1) water access difficulty, (2) area of forest plantations around farms, (3) cultivated homogeneity (crop diversity) and (4) farm location within the watershed. Drought response capacity was estimated by indicators such as (1) farmers knowledge of agricultural practices to withstand droughts, (2) conservation and use of drought-resistant crop varieties, and (3) water-related social networks. Indicators were assessed using a range of sampling techniques, including individual and group interviews, socio-economic surveys, landscape analysis using GIS tools, review of farm records and direct measurements on farms (Montalba et al., 2015). The influence of ethnicity was assessed using the Tukey HSD (Tukey Honest Significant Difference) *post-hoc* test. The presence of spatial autocorrelation in the values for each variable analyzed was assessed using the Mantel test with 999 iterations. All statistical analyses were performed using R v.2.15.0.

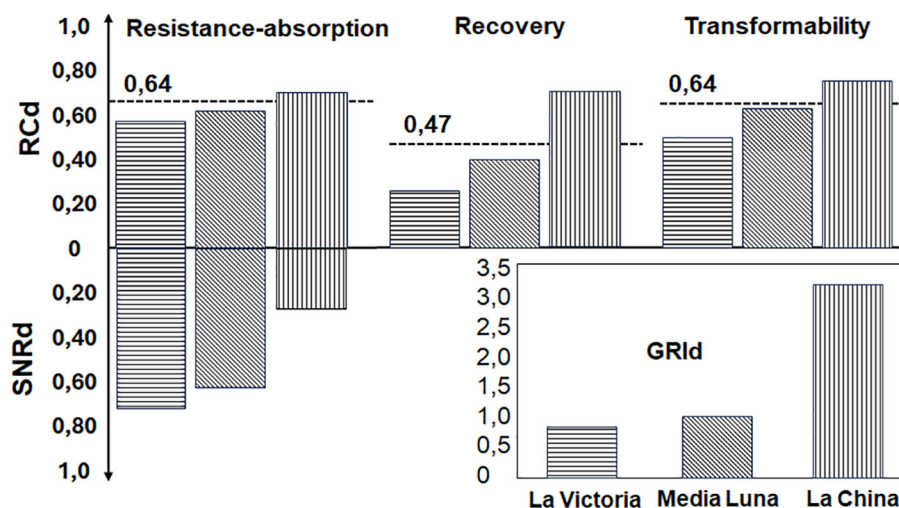


FIGURE 2

Summary of results from indicators applied to three suburban farms in La Habana, Cuba. Estimating Resilience Capacity to Drought (RCd), Sensitivity of Natural Resources (SNRd) and the General Drought Resiliency Index (GRId) (Vázquez et al., 2019).

The estimated resilience value was higher in Mapuche farms with a mean value of 0.88 (0,2.7) [optimal value around 1,5], while in Chilean and European farms resilience values were 0.52 (0, 1.38) and 0.55 (0,1.97) respectively. As observed in Figure 3, Mapuche farmers exhibited lower levels of vulnerability, possibly due to their lower proximity to pine/eucalyptus plantations in a radius of 1 km and greater crop diversity compared to Chilean and European settler farms. Mapuche farms also showed higher capacity to cope with drought, due to their command on various drought ameliorating practices and the use of tolerant crops and varieties. Chilean farms exhibited higher levels of water-related social networks, facilitating their access to declining water supplies, but the homogeneity of their agrolandscapes made them more vulnerable. Results suggest a greater resilience of Mapuche farming systems to drought, which is closely linked to their crop diversity, maintenance of traditional knowledge and practices and the conservation of local varieties and seed exchange.

The results underline the importance of agricultural biodiversity and traditional practices in improving resilience to climate change. Although modern agricultural policies often undervalue these systems, this study shows that traditional agricultural practices, rooted in indigenous and farmers' knowledge, contribute to the resilience of agricultural systems and to food security in times of hydric stress.

6 The limits of resilience

It is important to identify the limits of resilience before an agroecosystem subjected to an extended climatic stress reaches the

tipping points (thresholds) that lead to potential long-term or irreversible consequences (Huang et al., 2022). Observations in Central America and the Caribbean after recent hurricanes showed that in general agroecological farms coped better than conventional farms. However in areas with steeper slopes, the difference in agroecological resilience between diversified farms and conventional monocultures were less clear as the combination of rainfall intensity and slope became so great that differences in resilience between the two types of farms were no longer apparent. Although factors such as exposure, farm design and management practices mitigated impact, on average agroecological farms suffered as much damage as conventional farms (Holt-Giménez, 2002). Similarly in Cuba, highly diversified farms close to the coast, suffered high levels of damage due to their extreme exposure to rains, winds and sea penetration caused by Hurricane Irma (Vázquez, 2021).

In Puerto Rico, the resilience usually associated with the shade coffee systems was “cancelled” during the dramatic disturbance caused by Hurricane Maria in Puerto Rico, a phenomenon that may occur more commonly as climate change continues its course (Perfecto et al., 2019). Similarly, in areas affected by prolonged droughts and in the absence of irrigation, it doesn't matter how much organic matter is added to the soil to store water, or how much soil is covered with mulch to prevent evaporation, most crops succumb after a prolonged water stress (Tyagi et al., 2020).

This “cancellation of resilience” occurs when the severity and length of the climatic event pushes the agricultural system from one stable state to a deteriorating one. Determining the limits of resilience is not only key to assess impacts of climate change but it is also a precondition to define effective climate change adaptation strategies.

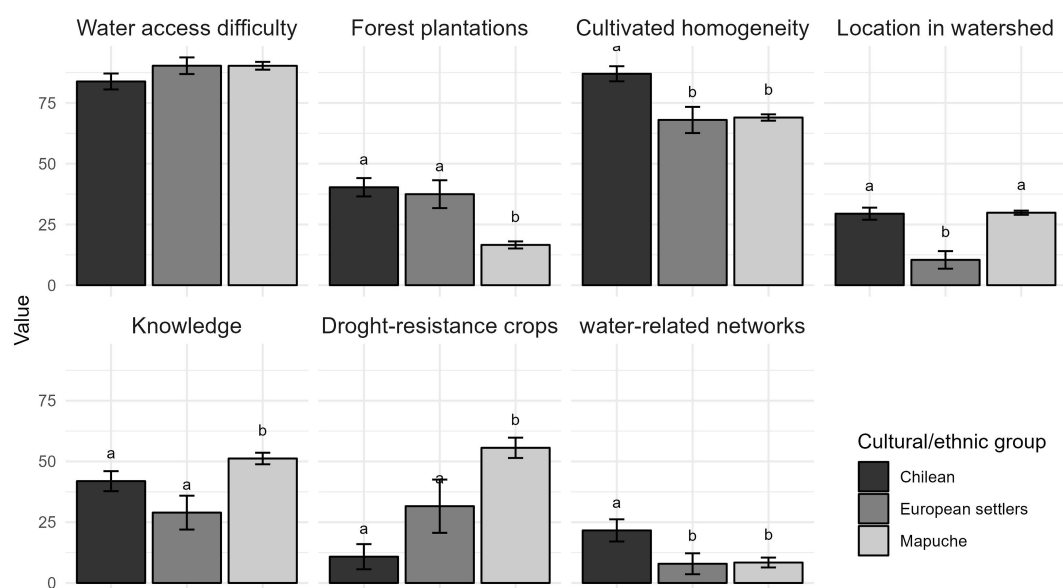


FIGURE 3

Variables used to estimate levels of vulnerability (first row of bar graphs) and drought response capacity (second row). In each bar graph, higher values indicate higher vulnerability to drought or greater drought response capacity in the various farmers grouped by cultural/ethnic origin in the Araucania region, Chile (Montalba et al., 2015). Lower-case letters are used to establish if the values represented in bars are or are not significantly different. Two bars with the same letter are not statistically different, bars with different letters are.

7 Extending the drought resilience limits

In rainfed farms affected by drought, a desirable range of soil moisture values should be maintained, in order for the system to continue functioning. It is important to set moisture limits for defined crop/environment situations, beyond which the system becomes unsustainable when it exceeds a designated trigger or threshold level (Morison et al., 2008). But more critical and of practical importance for farmers, it is to define whether a set of agroecological practices can extend such limits under prolonged water stress scenarios. In other words, is it possible to postpone the “resilience cancellation period”?

One key strategy is surface mulching which can optimize the partitioning of the water balance components, increasing moisture storage, leading to increased and water use efficiency (WUE) thus extending the crop cycle of low water requiring cropping systems (Lal, 1974). In most cases soil moisture content is directly linked to the degree of mulch cover. A study found that a 5 cm mulch depth minimized evaporation by 40%. An enhancement in mulch depth to 10 cm increased soil moisture by 10%, while a further boost (to 15 cm) provided no additional benefit. In north west India, straw mulching (6 t ha⁻¹) reduced soil water evaporation component of evapotranspiration (ET) by 18.5 to 23.8 cm in a range of crops, but it is not known how such reductions extended the crop growing period under drought (Jalota and Arora, 2002). One study found that zero tillage with residue retention buffered crops from short drought episodes and the extra 20 mm water that were available corresponded to the evapotranspiration requirements for 5 to 6 days of crop growth potentially extending the possibility of crop growth an extra 10–12 days in the absence of irrigation (Doorenbos and Kassam, 1979).

Under Mediterranean conditions, surface coverage with a mulch layer is an important water conservation practice with many studies reporting higher water storage over summer and decreased soil water evaporation, giving crop roots time to extract a greater proportion of the water from the surface soil. Soil water evaporation losses can be decreased over periods shorter than 14 days, provided that a 70%, or higher, shading is maintained through mulching practices. In order to obtain a 70% ground, cover a minimum of 6000 kg crop residue ha⁻¹ may be required (Beukes et al., 2004).

Mulching also improves root development leading to 30–50% gain in root weight compared to non-mulched crops. It is common to observe larger volume of root-permeated soil, enhanced lateral root extension and deeper root penetration after mulch application. Obviously extended and deeper root systems more fully explore the soil profile in search for hygroscopic water. Therefore, crops with deeper roots can better withstand a drought than crops with superficial root systems (Lal, 1978).

An unappreciated phenomenon is the fact that mulching positively influences soil biota, as soil cover improves

environmental conditions for soil organisms by increasing organic matter as a food source for microorganisms, invertebrates and earthworms. Straw and grass mulch significantly increased the amount and biomass of earthworms, organisms known to be effective in mixing the digested mulch material in the soil thereby improving soil structure and porosity. Researchers have observed maize roots to follow a stable worm channel to more than 120 cm depth. More lateral root spread under mulched strips was at least partially due to the sponge-like structure created by worm activity. It has also been observed that some mulches enhance naturally occurring mycorrhizae populations, and that water supply to crops is improved through mycorrhizal infection, allowing plants to better tolerate water stress (Jodaugienė et al., 2010).

Soils in dry climates have frequently low soil organic matter (SOM) content. Restoring soil organic matter can increase plant available water capacity in the root zone. Thus, addition of organic matter in the form of manure or compost, can significantly improve soil aggregation, macropores, lower bulk density and improve water retention and hydraulic conductivity (Magdoff and Weil, 2004). In fact, soils with low SOM content (0.5–1.0%) a 1% increase in SOM content in the 0–20 cm depth would increase available water to crops by 3–4 mm. For soils with higher SOM content 2–3% the available water increase would range from 1–2 mm, suggesting that the water storage effects of SOM are more effective in organic matter poor soils (Lal, 2020).

The available evidence indicates that the combination of mulching and SOM addition can increase plant available water capacity in the root zone and enhance a crop's tolerance to short-duration drought during the growing season (Zaongo et al., 1997). The effects of these strategies suggest that it is possible to extend the resilience limits but that long-term moisture conservation during prolonged dry periods may be less feasible. Clearly different agroecological practices have varied effects on soil water retention capacity. Table 1 presents a list of various adaptation measures available for farmers to cope with drought conditions. Based on current knowledge on the impact of each practice to ameliorate drought impacts (Sinclair et al., 2019; Seleiman et al., 2021) each practice is ranked according to its potential (high, medium or low) to extend the resilience threshold. Out of 15 practices, eight exhibit high potential to extend the limits of resilience to drought.

8 Conclusions

Climatic threshold refers to the levels of climatic factors (i.e. intensity and length of a drought) that can push an agricultural system from a relatively stable state to a deteriorating one. Determining the climate threshold for agricultural production under drought stress is not only key to assess climate change impacts but also to determine the types of adaptation strategies (Juhola et al., 2024).

TABLE 1 Potential of various agroecological practices in extending the limits of resilience to drought.

Practices	High	Medium	Low
Afforestation of field edges		✓	
Crop-animal integration		✓	
Crop rotation		✓	
Intercropping	✓		
Crop variety mixtures		✓	
Agroforestry	✓		
Timely sowing in climates where crop growth partially or largely coincides with a dry season.		✓	
Use of organic manure, compost, crop residues, etc.	✓		
Mulching	✓		
Planting of cover crops		✓	
Fallow practices			✓
Using seed coating to reduce risks associated with seed desiccation	✓		
Collecting water individually from roofs and catchment areas, water reservoirs, mini dams and wells	✓		
Introducing new, off-season vegetable production using water collected in wells and mini dams.	✓		
Applying a controlled amount of water for irrigation in key crop growth periods	✓		

✓ is used to denote if the practice has a high, medium or low impact in extending the drought tolerance.

The identification and assessment of current and projected future adaptation limits is essential for stabilizing food production with agroecological strategies. Resilience limits are likely to often be breached as droughts will become increasingly severe, widespread, and frequent. Current knowledge is far from understanding when and where limits will be reached and surpassed. Given such uncertainty, precautionary and transformational adaptation of agroecosystems requires a preventive approach based on agroecological principles.

Although there is an urgent need to adapt agroecosystems to changing climatic conditions, it is important to recognize the limits to such adaptation. Scientific evidence suggests that limits to adaptation may be extended beyond the established thresholds. The literature suggests that mulching and copious SOM applications can clearly extend crop growth periods under extended drought periods, but there is a limit if the event is too prolonged.

The adoption of some of the agroecological management strategies described herein allows farmers to offset impacts in a changing climate and are key to adaptations that can support livelihood outcomes such as food security by enhancing soil fertility, water retention, etc. These actions can enable farming systems to either recover to their previous state or evolve into more resilient systems. Either option, whether incremental (e.g., mulching or adopting cover crops) or transformative (e.g., transitioning from monoculture to diverse farming) is dependent on farmers' adaptive capacity—resources or assets farmers have access to, which play a key role in such decisions. To enable smallholders to reduce their exposure to drought and other hazards, new collaborative mechanisms beyond the farm scale are needed to optimize local hydrological cycles, for example, restoring local watersheds are necessary; but this implies major efforts to organize and engage in collective action.

The two methodologies described herein provide useful tools to assess the factors that determine the vulnerability of a particular agroecosystem to drought, and also to identify the response capacity of farmers to ameliorate the impacts. Both methodologies are simple enough to be used by farmers to assess whether their farms can withstand a drought and what to do to enhance the resiliency of the farm. The methodologies also help in identifying the principles and mechanisms that allowed certain farm systems to better resist and/or recover from droughts, which can be disseminated to other farmers via Campesino a Campesino exchange processes.

Indeed, farmers' personal resourcefulness, ingenuity and management skills (i.e. maintenance of traditional knowledge, use of efficient practices, etc.) help them to cope with the risk and uncertainty of natural disasters. However, when such events are compounded by uncertainties imposed by external economic conditions, such as input price increase for agricultural inputs or competition from imported foodstuffs, then farmers' abilities to draw on local knowledge and experience to pull them through adversity becomes much more problematic. The resilience of farms to climate disturbances can be diminished by rural conflicts unrelated to ecology, such as the expansion of palm, sugar cane and soybean monocultures and mining, which dry up streams and aquifers, which displaces the peasants. Addressing these broader agrarian issues suggests that promoting resilience in agriculture does not only consist of disseminating agroecological management, but also in confronting the inequalities and social injustices that afflict rural areas and transforming extractive agro-export economic systems into local and resilient food systems. To build resilience and prevent the next intense drought from becoming another catastrophe, it is necessary to scale up agroecologically based production models, but at the same time solve the underlying problems of access to land, water and seeds and the lack of markets and conducive policies that marginalize the peasantry, as well as challenging the corporate power that

controls food systems. These issue emphasize the importance of major political and socio-economic transformations including creation of enabling policies, a dimension beyond the scope of this review.

Author contributions

MA: Conceptualization, Writing – original draft. CN: Investigation, Methodology, Writing – review & editing. RM: Methodology, Writing – review & editing. LV: Methodology, Writing – review & editing. LV: Investigation, Methodology, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This study was partially funded by the Centro Latinoamericano de Investigaciones Agroecológicas (CELIA), Colombia.

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OPEN ACCESS

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RECEIVED 29 November 2024
ACCEPTED 25 February 2025
PUBLISHED 14 March 2025

CITATION
Negri L, Bosi S and Dinelli G (2025)
Agroecological strategies for innovation and
sustainability of agriculture production in the
climate change context: a comparative
analysis between California and Italy.
Front. Agron. 7:1536997.
doi: 10.3389/fagro.2025.1536997

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Agroecological strategies for innovation and sustainability of agriculture production in the climate change context: a comparative analysis between California and Italy

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Agriculture is profoundly affected by climate change, with regions like California and Italy experiencing significant challenges due to rising temperatures, altered precipitation patterns, and extreme weather events. Climate change is expected to reduce yields of specialty crops by up to 30% due to lower productivity and crop failure. To cope with climate change, farmers need to modify production and farm management practices, especially adopting agroecological principles. This mini-review explores climate change impacts on agriculture through an innovative approach that seeks to compare possible response strategies in two distant regions, California and Italy, which share similar climate conditions and crops. California's agriculture, renowned for its specialty crops like nuts, fruits, and vegetables, faces intensifying droughts, reduced snowpack, and increased potential evapotranspiration, threatening water availability and crop yields. Similarly, Italy, a Mediterranean climate change hotspot, endures higher temperatures, declining rainfall, and frequent extreme events, impacting key crops like grapes, olives, and tomatoes. Both regions see vulnerabilities compounded by climate-induced pest pressures and water scarcity. Agroecology emerges as a promising solution to mitigate these impacts by enhancing soil health, conserving water, and promoting biodiversity. Practices such as cover cropping, crop diversification, organic mulching, and precision irrigation bolster resilience. Site-specific strategies and policy support are crucial for adoption, especially in small-scale farms. Collaborative knowledge-sharing between California and Italy can foster innovative solutions, ensuring sustainable and resilient agricultural systems in the face of climate change.

KEYWORDS

resilience, soil health, drought, mediterranean climate, biodiversity, policy analysis, climate adaptation

1 Introduction

The anthropogenic causes of climate change have been scientifically demonstrated, resulting in an increasingly various pattern of meteorological and hydrological events around the planet, from heat waves to coastal flooding during extreme tides and storms, flooding from more intense precipitation events, and severe drought periods (Mann and Gleick, 2015; IPCC, 2023).

Industrial agriculture contributes significantly to climate change, especially in its release of methane and nitrous oxide from livestock and land use change (Clark et al., 2020). Agriculture, like all biological processes and human activities, is under siege from the impacts of climate change and in an unknown scenario (Ripple et al., 2023). Climate change may affect crops' productivity with changing precipitation and temperature patterns, but also leading to higher frequency in extreme events and exacerbating pest and disease pressure on crops (Burdon and Zhan, 2020). Warmer temperatures may favor some crop pests; besides, they can react differently to precipitations, depending on their exact timing and amount (Skendzic et al., 2021). Climate change may also increase or decrease weed pressure and incidence, depending on many causes and different weed-crop species combinations (Shahzad et al., 2021). Ironically, some of the most important agriculture regions of the planet are threaten by water-scarcity problems, especially in future years, such as the arid southwestern USA (e.g., California's San Joaquin and Imperial-Coachella Valleys) or the Mediterranean region (e.g., Italy) (Abd-Elmabod et al., 2020; Corwin, 2020). In fact, California and Italy represent two key agricultural regions with globally significant production but are extremely vulnerable to the impacts of extreme climate events and for these reasons they have been considered as relevant geographical areas for this study. This

mini-review discusses the climate change impacts on the agriculture sector of two different and very far geographical regions, California and Italy, which, however, share many climatic conditions and cultivated crop species (Figure 1). The present study seeks to highlight how the adoption of agroecological principles, adapted as site-specific farming practices, represents the real alternative to ensure climatically resilient agricultural production in future decades.

2 Climate change impacts on California agriculture

California is one of the most important and diversified agricultural regions of the world (Petersen-Rockney, 2022a). Around 50% of the nuts (such as almonds, pistachios or walnuts) and fruits (including grapes, citrus, apricots, dates, figs, kiwi fruit, nectarines, prunes, and olives) consumed in the United States (US) are cultivated in California (Pathak et al., 2018). Considering the high relevance and economic value of these specialty crops and their specific environmental growth requirements, agricultural production in California is highly sensitive to climate change impacts.

The cumulative co-manifestation of dry and warm years in the "Golden State" increases the risk of drought stresses, highlighting the significant role of high temperatures in modifying water availability and overall drought impacts on agriculture sector (Mann and Gleick, 2015). For these reasons, California represents a valid case study to explore how agriculture sector is impacted but can also react to climate change and climatic extreme events, especially drought conditions (Petersen-Rockney, 2022b). Extreme weather events in the State, including more frequent heatwaves, heavy and extended drought conditions, floods are negatively

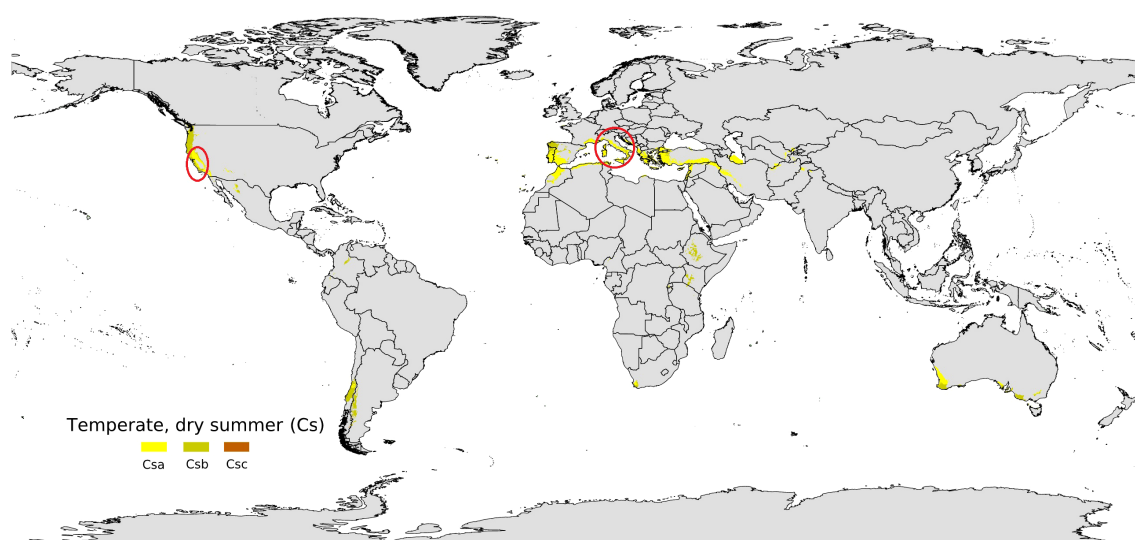


FIGURE 1

Global distribution of the Mediterranean climate (Cs) areas, following the Köppen–Geiger climate classification (Beck et al., 2018). California and Italy are marked in red among the main five global regions with this type of climate (California, Mediterranean basin, Chile, South Africa, Australia). Csa, Hot-summer Mediterranean climate; Csb, Warm-summer Mediterranean climate; Csc, Cold-summer Mediterranean climate.

impacting agriculture (Pathak et al., 2018; Weiskopf et al., 2020) and are estimated to increase in their intensity and frequency (IPCC, 2023). Analyzing California's climate data over the past four decades, autumn precipitation has decreased by 30%, while temperatures have increased by about 1°C (Goss et al., 2020). Average temperature increases projections predict that higher temperatures will be more evident during the summer season than in the winter and there will be more warming in inland areas than in coastal regions (Pathak et al., 2018).

Regarding future precipitation scenarios, California will maintain its Mediterranean climate with moderately cold and wet winters and hot dry summers (Pathak et al., 2018). Different general circulation models forecast that Northern California may experience higher annual precipitation amounts and probably more frequent storm events, while the overall state and especially Southern California are projected to be 15 to 35% drier by 2100 (DWR, C.D.o.W.R., 2015). In fact, almost 80% of the California's water in a typical year is provided by snow (Pathak et al., 2018). Provisional climate models suggest that 65% snowpack losses might occur by 2100, due to global warming (DWR, C.D.o.W.R., 2015). Generally, California's climate is shifting toward a flood-drought pattern, also resulting in increased flood risks (Pathak et al., 2018).

Even if climate change globally impacts have been in-deep studied for main field crops, major impacts in California are related to "specialty crops", defined by the United States Department of Agriculture (USDA) as all fruits (e.g. grape), nuts, vegetables (e.g. tomato), and nursery crops, which account for the highest economical production of Californian agriculture (Kerr et al., 2017). This unique relevance is possible because of California's Mediterranean climate (exclusive in North America) and the large-scale supply systems for irrigation water. Grapes and tomatoes represent more than 20% of California "specialty crops" value (Kerr et al., 2017) and their importance for the agricultural sector could be considered similar also in the Italian agriculture.

The majority of specialty crops in California is irrigated, and around half of this irrigation is provided by groundwater (Cooley et al., 2015). Even if irrigation water could disguise the impacts on yields of climate change, potential evapotranspiration in California's specialty crop growing regions will significantly increase, according to the future climate scenario (Kerr et al., 2017). Already for several years and more and more now, there are increasing concerns about whether California can continue to satisfy its massive water demand for industrial purposes, agriculture production, preserving ecosystems, and developing cities in the midst of drought (Christian-Smith et al., 2015).

Besides worsening pathogens or insect pests (Trumble and Butler, 2009; Jha et al., 2024) pressure on crops, driven by climate change, it's estimated that, with a global warming trend of 3°C, weed species pressure in California and the central Midwest will substantially increase, for example considering itchgrass or witchweed (Anwar et al., 2021). Considering that the profitable value of specialty crops production is not simply related to yields but also to several quality characteristics (for example aesthetic features, shape, size or chemical composition), the majority of Californian agriculture production is particularly susceptible to climate change impacts (Pathak et al., 2018).

3 Climate change impacts on Italian agriculture

Mediterranean countries, such as Italy, have been recognized as climate change "hot spot", since the incidence of high temperature extremes is estimated to increase by 200 to 500%, considering future greenhouse gas emissions scenarios (Nikolaou et al., 2020). The effect of climate change in Italy is increasingly perceived by citizens. In 2021, a Eurobarometer analysis highlighted that climate is the fourth concern for Italian citizens, following diseases, economy, and world hunger (De Leo et al., 2023). Similarly to California, drought is a raising challenge for Italy's agricultural sector, causing a problem for the country's major crops, as well as smaller farmers (OECD/FAO, 2021).

Average temperatures in the Mediterranean region are rising faster than the global average (Dari et al., 2023). Moreover, rainfall across the region is expected to decrease by 10% to 60% (Dari et al., 2023), exacerbating water scarcity issues, crucial for Italy's water-intensive crops like rice and corn (Straffellini and Tarolli, 2023). Droughts, like those observed in recent years, have already caused significant yield reductions, while sudden storms and hail have damaged vineyards and olive groves, two pillars of Italian agriculture (Aguilera et al., 2020; Santos et al., 2020).

The largest decreases in productivity for Italy are expected for crops with a spring-summer cycle, especially if they are not irrigated, with yield reductions especially for corn, sunflower and sugar beet, while slight increases are expected for wheat (Hristov et al., 2020). Webber et al. (2018) reported that heat stress does not increase for corn and wheat crops under non-irrigated conditions, while water stress only intensifies for corn (with yield decreases in Italy around -20% values) and not for wheat (which instead shows stable yields or even increases of up to +20% in some areas of the country). Declines in rainfall directly impact crop yields. Corn, a major crop in northern Italy, relies on consistent irrigation, which is now threatened by shrinking water supplies from rivers like the Po river (Hristov et al., 2020). The projected raise in air temperature and changes in rainfall may cause a shortening ranging from 1.5 to 3 days in tomato phenology, triggering an overall 15% reduction in tomato yield (Camarano et al., 2020).

Among tree crops, grapes, essential for Italy's globally renowned wine sector, are highly sensitive to temperature changes. Some regions may need to adapt by shifting vineyards to higher altitudes or adopting heat-resistant varieties, in order to maintain production and quality standards (Droulia and Charalampopoulos, 2021). Olive trees, resilient to drought, are now facing challenges from rising temperatures and the proliferation of pests like the olive fruit fly, which thrives in warmer climates (Aguilera et al., 2020).

The economic levy of climate change on Italian agriculture is significant, with damages from extreme weather estimated at over €14 billion in the last decade (De Leo et al., 2023). Climate change disrupts rural livelihoods, reducing employment opportunities and exacerbating rural depopulation. Small-scale farmers, who dominate the Italian agricultural landscape, are particularly vulnerable due to limited resources for adaptation (De Leo et al., 2023).

4 Agroecology and climate change resilience in California and Italy

Agroecology, integrating ecological principles into agricultural practices, offers a promising path to strengthen climate resilience by enhancing soil health, water efficiency, and ecosystem services (Altieri et al., 2015). Agroecology provides the best agricultural approach capable of coping with future challenges, by promoting high levels of diversity and resilience, while producing acceptable yields and ecosystem services (Altieri and Nicholls, 2020). Agroecology promotes the regeneration of the landscapes in which farming systems are present, improving the ecological networks, that may help in pathogens and pests prevention (Altieri and Nicholls, 2004).

California farmers will be challenged to adopt adaptation strategies in the future. In fact, California's agriculture faces significant threats from climate change, particularly due to intensifying droughts and extreme weather events.

On the other side of the Atlantic Ocean, the vulnerability of Italian agroecosystems is a specific component of total changes affecting the Mediterranean basin, characterized by biodiversity loss, freshwater overemployment, disturbed nutrient cycles, soil losses and different fire patterns. This context is exacerbated in Italy by conditions of high population density, water scarcity, high dependence on material and energy imports, combined with the predominance of highly specialized and poorly diverse agroecosystems (Aguilera et al., 2020). Due to the need to create resilience to these connected risks, systemic adaptation measures are straightaway needed (OECD/FAO, 2021). Agroecology is based on an holistic vision, enabling the recovery and valorization of traditional knowledge and the co-creation of new local knowledge, for enhancing resilience (Aguilera et al., 2020).

4.1 Agroecological strategies across regions

4.1.1 Agroecology, healthy soil and water

Healthy soils are critical for water retention and drought resilience. Practices like cover cropping, reduced tillage, and compost application, applied in tree and vegetable crops, very important in California and Italy, can increase soil organic matter, enhancing its capacity to hold water (Teng et al., 2024; Diacono et al., 2016). Studies have shown that these methods improve the water-holding capacity of soils up to 30%, crucial for sustaining crops during prolonged dry periods, frequent in California and Italy (especially in the Southern areas of the country) and reduce soil erosion, essential action for steep terrains (van Zonneveld et al., 2020; Pagliacci et al., 2020). Water scarcity, exacerbated by declining rainfall and shrinking snowpack, is a critical challenge for Italian agriculture. Drip irrigation and other precision systems, often used in agroecological settings, deliver water directly to roots, reducing losses by up to 40%, compared to traditional irrigation (Nikolaou et al., 2020). Combined with techniques like rainwater harvesting and the use of drought-tolerant crop varieties, these approaches help

maintain productivity during prolonged dry periods (Altieri and Nicholls, 2017; Santos et al., 2020). In Mediterranean conditions, such as Italy's agricultural main areas, adopting mulching alongside efficient irrigation reduced evaporation rates, enabling farmers to meet crop water needs, with 20% less water during drought periods (OECD/FAO, 2021; Romero et al., 2022).

4.1.2 Agroecology and biodiversity

Diversifying crops through polycultures or intercropping can stabilize yields, by spreading risk across different species, with adjusted drought and heat tolerances, even if the yields could be lower in short-term time scale (Petersen-Rockney, 2022b). Furthermore, agroecology promotes natural pest control, reducing reliance on chemical inputs that may exacerbate water pollution and biodiversity loss (Carlisle et al., 2022). Practices such as agroforestry and managed grazing improve groundwater recharge and reduce surface evaporation, increasing soil water content up to 20%, depending on the specific pedo-climatic conditions and the cultivated crops (Belmin et al., 2023). For example, planting deep-rooted perennials alongside annual crops can optimize water uptake across soil layers while providing shade and reducing heat stress on plants. Crop diversification tends to stabilize yields by spreading the risk of failure across multiple species with different drought and heat tolerance (Altieri et al., 2015; Smith et al., 2023). For instance, polycultures, including legumes intercropped with cereals, improve nitrogen fixation by approximately 10–15% and reduce the vulnerability of monocultures to extreme weather events (Książek et al., 2023). These systems are particularly effective in Italy's arid southern regions, where climatic variability is high. As an additional diversification strategy, adopting agroforestry in Italian farms may contribute to create microclimates that reduce heat stress on plants and prevents soil erosion, with temperatures in the field crops areas between trees about 0.5–1.0°C lower than the monoculture (Piotto et al., 2024; Romero et al., 2022).

4.1.3 Agroecology and landscape

Moreover, agroecology strengthens resilience against extreme heat and storms by fostering adaptive landscapes. Windbreaks and shelterbelts protect crops from strong winds and reduce topsoil erosion during storms (Parker et al., 2023). Deep-rooted perennials, such as certain fruit trees, are better adapted to extreme weather fluctuations, offering consistent productivity under climate stress (Parker et al., 2022). Research underscores the importance of site-specific practices, as climatic conditions and soil types vary widely across California. For instance, increasing the resilience of high-value crops like almonds and tomatoes demands tailored approaches that combine agroecological methods with advanced irrigation systems (Pathak and Stoddard, 2018; Parker et al., 2022). Agroecological landscapes are inherently more resilient to storms and extreme rainfall. After intense rain events in central Italy, farms employing agroecological practices such as minimum tillage, organic mulching based also on crop residues, permanent plant soil cover, reported up to 60% less soil loss compared to conventional systems (Kassam et al., 2012; Napoli et al., 2017). Climate change intensified pest and disease pressures in Italy, but

agroecology contributes to maintain ecological balance, by harnessing natural predators (Bindi and Olesen, 2010; Scotti et al., 2023). Hedgerows, flower strips or cover cropping promote the presence of beneficial insects, reducing reliance on pesticides. Studies conducted in Italy demonstrated a reduction in pest populations in common wheat or vegetable crops, adopting such practices (Magagnoli et al., 2018, 2024).

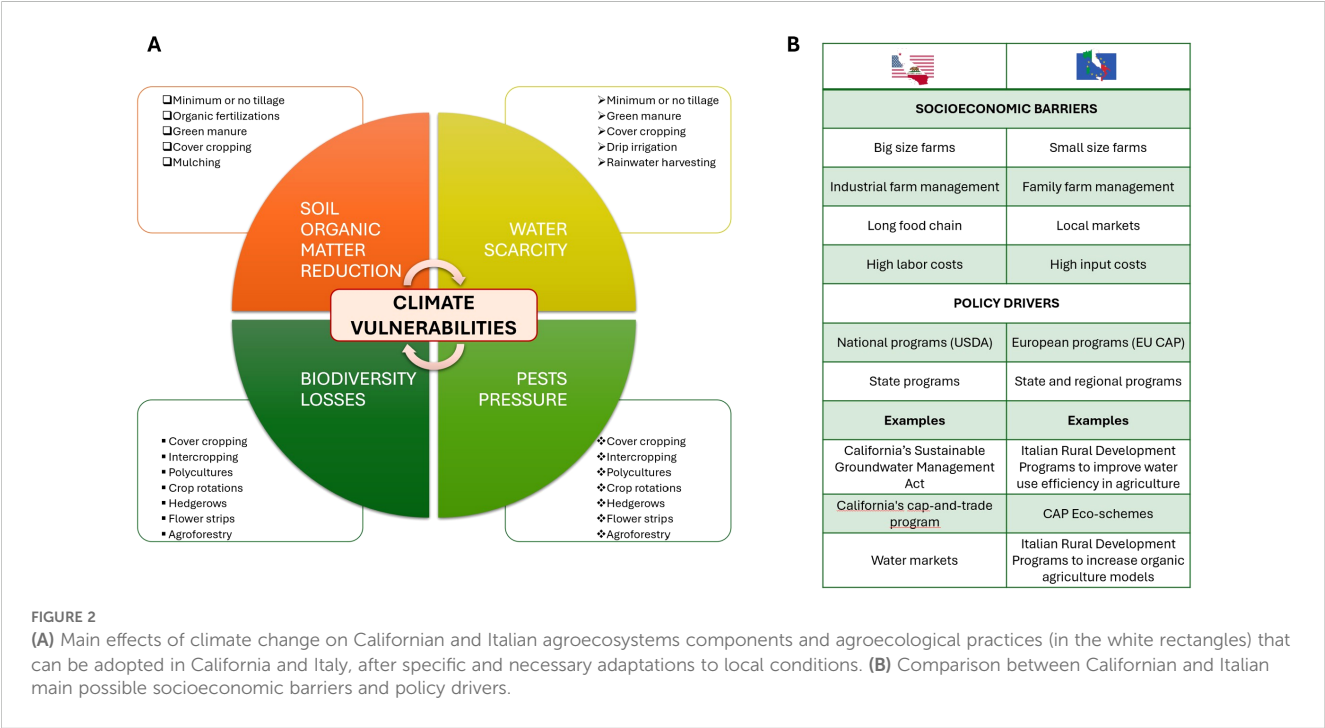
4.1.4 Agroecology, policies and socioeconomic influences

Further, funding mechanisms and extension services are pivotal in promoting agroecology. Policies that support conservation tillage, diversified cropping systems, and organic farming can encourage widespread adoption, improving agricultural sustainability across California (Carlisle et al., 2022; Belmin et al., 2023). In California’s corporate agribusiness farming structure, strengthening knowledge exchange among farmers and supporting local farmers’ initiatives can potentially contribute to the diffusion of agroecological practices (Kreft et al., 2023). California government support farmers to incur the high investment costs and reduce GHG emissions to adapt to water restrictions by directly funding the modernization of underground water pumps and the installation of drip or micro sprinkler irrigation systems (Zhao et al., 2023). By fostering biodiversity, improving soil health, and optimizing water use, these practices not only mitigate the impacts of drought and extreme events but also contribute to long-term overall sustainability (Teng et al., 2024). In Europe, citizens tend to pay more attention to the impacts of climate change on agricultural development (Zhao et al., 2023). As a consequence, many different strategies, initiatives, and regulations related to support agroecological approach and practices have been developed at the regional (e.g. Italy’s Rural

Development Program), national (e.g. Organic National Regulation), and European (e.g. Common Agricultural Policy - CAP, European Green Deal - EGD) levels (Francaviglia et al., 2023). Both the CAP and the EGD should preserve ambitious environmental commitments to avoid additional losses of the natural resources on which agroecosystems rely. These include proportional allocation of funds to each CAP goal, quantitative objectives and appropriate indicators to facilitate useful monitoring of environmental performances (Cuadros-Casanova et al., 2023). In Italy, policymakers must support agroecological practices through funding, research, and farmer education programs, considering also the fact that some agroecological practices can be labor-intensive and Italian farms tend to be small and managed by old farmers, often not well-integrated into profitable value chains (OECD/FAO, 2021). This characteristic does not facilitate a change in the agronomic management models, aimed to reduce the use of external inputs and to adopt agroecological practices that will increase the resilience of agroecosystems, highly threatened by the impacts of climate change, such as those in Italy.

4.2 Conclusion

Farmers in California and Italy are experiencing increasingly extreme climatic events (Pathak et al., 2018). At the same time, the accelerating rate and the increasing scale of climate impacts, including novel droughts and water excess conditions, reduce farmers’ capacity to adopt conventional agricultural practices (Petersen-Rockney, 2022a). The adoption of alternative and site-specific practices and strategies, based on agroecological principles, will represent a successful reaction to climate impacts, both in California and Italy scenarios (Bezner Kerr et al., 2023). This study



clearly shows the importance of international and local cooperation, especially through the exchange of knowledge and practices between regions facing similar challenges but that can react with different and local optimized practices (Figure 2). The scientific and technical cooperation, together with rational public policies, can represent a winning strategy to address the climate impacts on agriculture.

Author contributions

LN: Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review & editing. SB: Supervision, Writing – review & editing. GD: Writing – review & editing, Supervision.

Funding

The author(s) declare that no financial support was received for the research and/or publication of this article.

Acknowledgments

This mini-review would not have been possible without the exceptional support of Prof. Miguel A. Altieri and Prof. Clara I.

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RECEIVED 29 November 2024

ACCEPTED 14 March 2025

PUBLISHED 08 April 2025

CITATION

Kuria AW, Pagella T, Muthuri CW and
Sinclair FL (2025) Revisiting agroecological
transitions in Rwanda a decade later:
the role of local knowledge in
understanding the crop diversity–food
security–land degradation nexus.
Front. Agron. 7:1537012.
doi: 10.3389/fagro.2025.1537012

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Revisiting agroecological transitions in Rwanda a decade later: the role of local knowledge in understanding the crop diversity–food security–land degradation nexus

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The challenge of achieving food security amidst broken food systems, the climate crisis, biodiversity loss, degrading land, and growing social inequity remains a critical development priority in alignment with the Vision 2030 agenda. While crop diversification is a cornerstone of agroecological transitions and food security, global food systems have often overlooked its potential, largely due to insufficient local participation and the reliance on blanket policies unsuitable for heterogeneous contexts. This article revisits agroecological transitions in Western Rwanda a decade after data collection, assessing the enduring relevance of local knowledge in understanding the crop diversity–food security–land degradation nexus. Using a systematic knowledge-based approach (AKT5), data were collected from 150 smallholder farmers through a Paired Catchment Assessment. Findings from the 1995–2015 period revealed a decline or disappearance of “low-value” crops, driven by the Crop Intensification Program (76%), land shortages (55%), and abandonment of slow-growing crops (49%). As a result, 83% of farmers reported food insecurity, primarily manifesting as seasonal food shortages (51%). Perennial crops emerged as critical for bridging hunger gaps, while reduced crop diversity forced many farmers to rely on off-farm food sources. The original analysis identified seven agroecological principles integral to the crop diversity–food security nexus: soil health, biodiversity, synergy, economic diversification, social values and diets, co-creation of knowledge, and participation. These findings varied significantly by land degradation status, emphasizing the importance of context-specific solutions. This study also showed that farmers have become more dependent on sourcing food off-farm, with food produced on-farm supporting farmers for an average of 6.6 months annually in 2015 compared to 10.1 months in 1995. This underpins the need to leverage ecological rather than administrative boundaries, ensuring connectivity within food systems, and fostering equitable trade mechanisms for smallholder farmers if agroecological transitions are to be realized. A decade later, the findings of this study were reflected upon and validated through recent literature, which underpins the

validity of local knowledge in understanding of agroecological transitions. This advocates for stronger integration of local knowledge, stakeholder collaboration to promote the co-design of tailored context-appropriate, inclusive, and sustainable policy frameworks to foster sustainable food systems across scales.

KEYWORDS

local knowledge, crop diversity, agroecology, food security, land degradation, smallholder farmers, knowledge co-creation

1 Introduction

Crop diversity plays a critical role in steering agroecological transitions towards meeting the various dimensions of food security needs across heterogeneous and multi-functional agricultural ecosystems. Sustainable agriculture and food systems that are achieved through agroecology simultaneously offer multiple benefits to society (Nicholls and Altieri, 2018). This is because agroecology promotes a shift from generalized to customized production systems and promotes ecological, social, economic and nutritional diversity of systems (Wezel et al., 2020). Agroecological approaches including principles and practices thus utilize comprehensive ecological, economic, and social principles in the transition of small-scale farming systems, with the aim of enhancing their resilience (Savels et al., 2024; Ume et al., 2023). This involves tailoring 13 universal agroecological principles (recycling, input reduction, soil health, animal health, biodiversity, economic diversification, social values and diets, fairness, connectivity, land and natural resource governance; and participation) to suit specific local conditions (HLPE, 2019; Sinclair et al., 2019).

Multifunctionality of agricultural systems is enhanced through the increased functional diversity of crop polycultures (Cordeau, 2024; Finney and Kaye, 2017). Agroecological ecosystems comprising of diverse crop species produce multiple ecological goods and services and contribute to their continuous regeneration and resilience compared to less diverse systems (Kahiluoto, 2020; Matsushita et al., 2016). There is evidence that intercropped systems are more ecologically and socio-economically resilient compared to monocrops (Bowles et al., 2020). Combinations of crops is thus beneficial as it contributes significantly to ecological synergies as each crop performs a specific function within the agricultural ecosystem and also results into beneficial interactions amongst crops being grown (Franco et al., 2015). Further, not only is diversity critical at the species level but also at the genetic level as crop genetic diversity leads to long-term agroecological resilience and stability of ecosystems such as through climate-resilience and pest and disease resistance (Jacques and Jacques, 2012; Sanya et al., 2020).

Integrating perennial crops with annual crop species is a particularly effective strategy for increasing on-farm crop diversity. This ensures that while annual crops provide short and

mid-term services such as food, feed and fuel; perennial crops can provide long-term multiple environmental services such as soil nutrient cycling, carbon sequestration, ground water recharge, pest and disease control and enhanced crop pollination (Bowles et al., 2020; Muthuri et al., 2023). Ndoli et al. (2021) found a positive correlation between perennial crop diversity and food security. Different perennial crop species have for example been found to favor different beneficial soil macrofauna species (Kamau et al., 2017). This includes facilitating soil aggregation resulting from enhancing soil microbial community composition (Tian et al., 2019). When it comes to perennial crops, Endale et al. (2017) notes that for systems to operate optimally and in order to generate sufficient ecological goods and services, there is need to not only increase species richness but also abundance.

In Rwanda like in most rural sub-Saharan Africa communities, where smallholder farmers rely on agriculture for their livelihoods, food security is closely tied to crop diversity. Empirical studies suggest that diverse cropping systems contribute to food security by enhancing availability, access, utilization, and stability (Mango et al., 2018; Mengistu et al., 2021). Furthermore, crop diversity has been closely linked to dietary diversity, providing essential micronutrients that improve health outcomes (Nicholson et al., 2021; Rajendran et al., 2017). Despite high crop diversity playing a key role in steering agroecological transitions towards meeting through enhancing food security, productivity and resilience of agricultural systems (Bourke et al., 2021), majority of development efforts in sub-Saharan Africa countries including Rwanda have mostly focused on enhancing productivity and closing yield gaps of a few selected mono-crops (Kim et al., 2022; Schrama et al., 2018). While majority of largescale farms across the world are simplified by monocrops, majority of smallholder farms especially in sub-Saharan Africa are mostly characterized by complex and diverse cropping systems (Osahar and Allan, 2003). Studies have shown that smallholder farms are highly heterogeneous ecologically, social-economically, biophysically, historically and politically (Kuriah et al., 2024; Vanlauwe et al., 2014). Hence the systems hold varying crops and crop diversity trends; and populations experience different types and levels of food insecurity and have varying vulnerability levels. Agriculture and food systems thus need to adapt to different contexts by adopting agricultural management practices to enhance crop diversity.

Secondly, despite food insecurity being multifaceted and drivers originating from multiple scales (Marchetti et al., 2020), majority of policy makers have often designed food security policies at coarse scale, either at the global, regional or national level (Duncan et al., 2022; Lele et al., 2013). Majority of food security metrics and indicators used are often generated through top-down approaches that are generalized across heterogeneous landscapes. Top-down coarse approaches take away the target population, who understands their local agroecological system intricately, from being part of solutions aimed at improving food security (Duncan and Claeys, 2018). This results in inappropriate, unsustainable and skewed interventions and the inability to meet all the dimensions of food security (Burchi and De Muro, 2016; De Haen et al., 2011). This leads to lack of customization of food security policies and programs to local context, which is mainly caused by the lack of co-creation of knowledge and failure to incorporate knowledge of local food producing communities in understanding the target context for which food policies and programs are being designed.

The urgency to adapt agricultural systems to current and emerging challenges—such as land degradation, climate change, population pressures, and disruptions like the COVID-19 pandemic—has heightened calls for agroecological transitions (Jha et al., 2021; Kumar et al., 2021). These transitions emphasize the need for context-appropriate policies that integrate local knowledge and address the specific needs of diverse communities, including marginalized groups such as women and children (de Araújo Palmeira et al., 2020; Singh et al., 2020).

Local knowledge refers to locally derived understanding which is based on experience and observation; and it is usually a mixture of traditional knowledge, knowledge acquired from external sources (education, media, dialogue with other communities) and contemporary learning (Dixon et al., 2001). Unlike scientific knowledge, which is often formalized and generalized, local knowledge is embedded in social structures, oral traditions, and cultural contexts (Agrawal, 1995). It is dynamic and evolves through experiential learning and adaptation to changing conditions, such as climate variability and shifting agricultural policies (Chambers, 2012). The process of translating local knowledge into scientific discourse is not merely an act of documentation but involves interpretation and contextualization to ensure that indigenous meanings and practices are preserved (Smith, 2012).

There is wide agreement on the need to change the prevalent generalized agricultural models, given their negative impacts and their incompatibility with current societal needs and dynamic context. There have been many calls for an agroecological transition to respond to food shocks and crises resulting from conventional generalized food systems to context-appropriate food systems (Sinclair et al., 2019). Agroecological transition has been promoted as a potential solution to the ecological, social and economic problems generated by these models. However, there is limited knowledge on the role of local knowledge in understanding the complex role that crop diversity plays in the context of food insecurity from an agroecology perspective.

Therefore, the overall objective of this study was to co-create knowledge on crop diversity and food security by integrating local knowledge with scientific perspectives. This study revisits agroecological transitions in Rwanda, a decade after data collection, to assess the effect of changes in crop diversity on food security. Specifically, it addresses three key hypotheses: (1) on-farm crop diversity has decreased over time, influencing food security status; (2) local knowledge enhances understanding of agroecological principles related to the crop diversity–food security nexus along a land degradation gradient; and (3) farmers have become increasingly reliant on off-farm food sources. By revisiting these dynamics, the study provides insights into the validity and role of local knowledge in designing adaptive, agroecological strategies for food security and sustainability in the face of evolving challenges.

2 Materials and methods

2.1 Study area characterization and selection

This study was undertaken in Gishwati, which falls under Rubavu and Nyabihu Districts of Western Rwanda. Gishwati area is known as Rwanda's food basket due to its sub-humid agroecological zone and rich volcanic soils which makes the area favorable for agriculture (Kabirigi et al., 2017; Kuria et al., 2019). Gishwati forest used to extend towards Lake Kivu at the Border of Rwanda and DRC but currently the forest consists of fragments resulting from deforestation whose drivers were three-fold namely: forest conversion to agricultural land for enhanced food security, settlements and over-extraction of tree products for building and fuelwood for returnees and refugees following the 1994–1995 genocide (Ordway, 2015).

Rubavu and Nyabihu districts are characterized by diverse agroecological conditions and socio-economic structures that influence farming systems and resource management. Both districts face land fragmentation due to high population density, with most farmers cultivating smallholder plots averaging less than 0.5 hectares (National Institute of Statistics of Rwanda, 2022). Land tenure systems include a mix of customary and formal ownership, with increasing formalization through land registration programs. In terms of gender roles, agriculture is the primary livelihood activity in both districts, with both men and women actively engaged. However, women face structural barriers to land ownership and decision-making within agricultural value chains because they often have limited control over land despite their significant role in farming activities, post-harvest processing, and household food security (Uwizeyimana et al., 2021). Gendered labor division also influences access to agricultural resources, training, and markets.

Given its proximity to the DRC, Rubavu has a dynamic agricultural economy, with a mix of subsistence farming and commercial activities. Farmers engage in small-scale trade, particularly in food crops and

livestock products. Urbanization and tourism contribute to diversified income sources (Ministry of Finance and Economic Planning, 2020). On the other hand, Nyabihu district is known for high-altitude farming, with a focus on potatoes, dairy production, and agroforestry systems. Limited road infrastructure and market linkages affect farmers' ability to commercialize surplus produce. Government and NGO interventions promote climate-smart agriculture and sustainable land management practices (Rwanda Environment Management Authority, 2019).

This research adopted a Paired-Catchment experimental design (Brown et al., 2005) and focused on three landscapes namely (Degraded, Recovering, Restored). We hypothesized that land degradation status heterogeneities present different sets of biophysical opportunities and challenges for crops and food security, hence unique entry points for agroecological practices (Nkheloane et al., 2012). Hence including landscapes under different degradation status would inform the design of more inclusive and diverse food security options. Historical timelines revealed that although all three study sites underwent simultaneous tree cover loss after the 1994–1995 genocide, they underwent different trajectories of land degradation and restoration (Aynekulu et al., 2014; Bigagaza et al., 2002). The topography of all sites is hilly with steep slopes (some areas have a slope inclination of over 50%), hence the landscape is susceptible to severe soil erosion (Byiringiro and Reardon, 1996; Kagabo et al., 2013). Due to the hilly nature of the landscape, the study thus

further stratified each landscape according to slope gradient, which included upslope, midslope and downslope farms. The degraded landscape was characterized by Alisols, which due to their poor structural stability and susceptibility to leaching and runoff are more prone to erosion than Andosols which have a well-aggregated structure (Food and Agriculture Organization, 2015; IUSS Working Group WRB, 2022), which were the dominant soils in the recovering and restored landscapes. The Recovering and Restored landscapes were adjacent to each other and neighboring Karago Lake and were located in Kadahenda cell, Karago sector of Nyabihu district (Figure 1). The Recovering landscape, whose study villages were Karandaryi, Gakoma and Nkomane, falls under the Eastern Congo-Nile Highland Subsistence agro-farming-ecological zone and lies between 2350 and 2540m.a.s.l. with average annual rainfall of 1200–1500mm. It is characterized by alisols and still experiences slight soil loss through surface run-off because it has more recent erosion control interventions (2012) compared to the Restored landscape (2007). The Recovering landscape is receiving soil and water conservation interventions and food security interventions implemented through the Trees for Food Security Project led by the World Agroforestry (ICRAF) through funding by the Australian Centre for International Agricultural Research (ACIAR) and has progressive terraces with trees and other vegetation planted along (Cyamweshi et al., 2021). The project aimed at sustainably improving productivity of farming landscapes, and to recover food

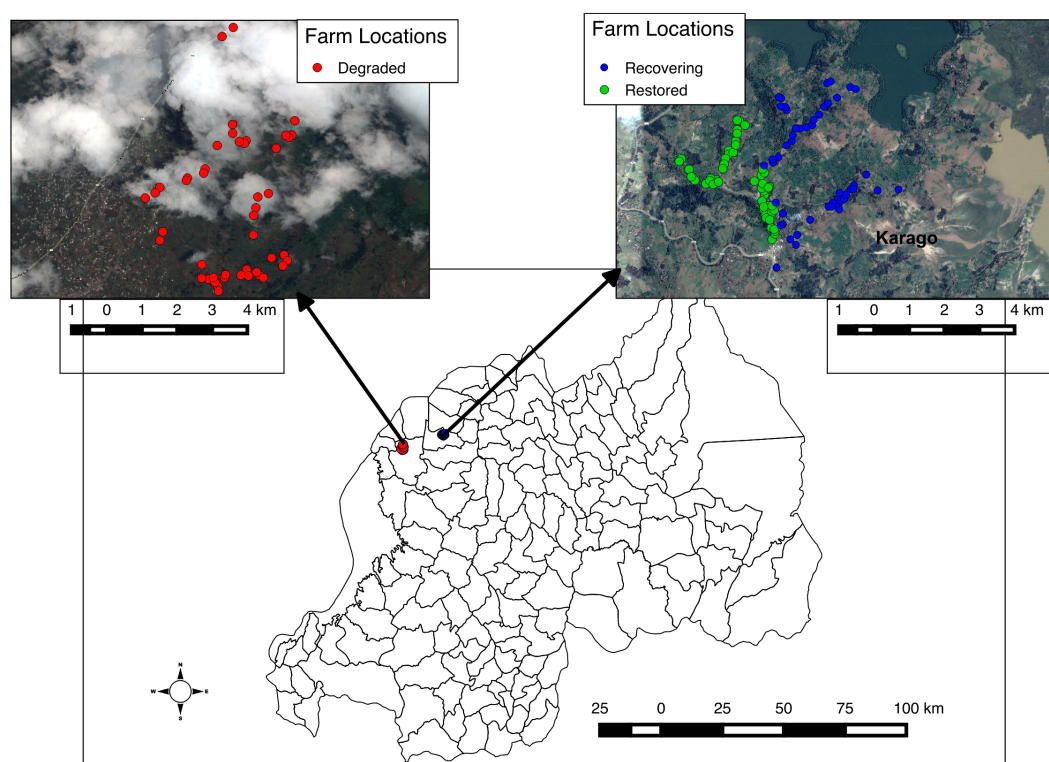


FIGURE 1
Map of Rwanda showing location of fields sampled in Gishwati.

and nutritional security through the promotion of suitable agroforestry interventions.

The Restored landscape (the study village was Gihira), falls under the Eastern Congo-Nile Highland Subsistence agro-farming-ecological zone and lies between 2380 and 2570m.a.s.l. with average annual rainfall of 1200–1500mm. It is characterized by alisols and soil loss had been controlled through soil and water conservation interventions implemented from 2007 namely bench and progressive terraces with vegetation planted along. In 2005/2006, the government of Rwanda through the ‘umuganda’ community service embarked on soil erosion control as part of the national soil and water conservation program; whereby bench and progressive terraces were established on steep slopes (Bizoza, 2014) and stabilized through planting of *Alnus acuminata* and *Setaria sphacelata*. The interventions were also meant to protect Lake Karago and Busoro river from siltation. In addition, the government set aside 50 meters of land adjacent to the water bodies for planting trees.

The Degraded landscape was in a different farming system located in Gikombe cell, of Nyakiliba sector in Rubavu district. The study villages were: Rushubi, Nyabibuye and Nyakibande. The landscape falls under the North-Western Volcanic Irish Potato Zone, between 1890 and 2180m.a.s.l. with average annual rainfall of 900–1500mm, is characterized by volcanic andosols and has no soil erosion control interventions hence it is characterized by severe soil loss as a result of soil erosion, landslides and siltation as well as frequent flooding in the downslope flat areas. The area has not received any soil and water conservation interventions following the post genocide deforestation in 1995. The upper part of the Degraded landscape is adjacent to Gishwati protected forest while the bottom part borders Mahoko town. After the government of Rwanda evicted farmers who had encroached Gishwati forest in 2010, and soil and water conservation efforts have mainly involved reforestation of the protected forest, and not the adjacent farming landscape, which was the focus for this study.

2.2 Data collection using the agroecological knowledge toolkit methodology

This study adopted a qualitative approach to assess the role of local knowledge in agroecological transitions, particularly in relation to crop diversity, food security, and land degradation. Data were collected through semi-structured interviews, focus group discussions, and field observations with smallholder farmers in Gishwati, Rwanda. The research framework is informed by the 13 agroecological principles proposed by the High-Level Panel of Experts on Food Security and Nutrition (HLPE, 2019), which serve as a guiding framework for transitioning towards sustainable food systems. These principles encompass ecological, socio-economic, and governance dimensions critical to agroecological transformations. During data analysis, we systematically examined how local knowledge aligns with these 13 agroecological principles. Rather than addressing all 13 principles in

detail, we identified seven principles that emerged as most relevant to the crop diversity–food security–land degradation nexus based on farmers’ experiences and responses. This analytical approach ensures that the findings remain empirically grounded while providing insights into the specific agroecological principles that shape sustainable food system transitions in Gishwati. Accordingly, the discussion section presents these seven principles, highlighting their significance in leveraging local knowledge for agroecological sustainability.

The study employed the AKT5 methodology, a knowledge-based systems approach that systematically integrates quantitative and qualitative research methods to systematically capture and analyze farmers’ knowledge on crop diversity and food security (Dixon et al., 2001). While it dates back to the late 1990s and early 2000s (Sinclair and Walker, 1998), AKT5 remains one of the most robust tools for capturing complex, context-specific knowledge systems related to agroecology. This methodology was thus chosen because it allows for structured knowledge elicitation while preserving the richness of farmers’ contextual experiences. AKT5 facilitates co-creation of knowledge by combining structured interviews and hierarchical knowledge organization, ensuring that insights from diverse farmers are systematically documented (Walker and Sinclair, 1998). While alternative methodologies such as ethnographic approaches (Agar, 2006; Turner and Berkes, 2006) or participatory rural appraisal (PRA) (Chambers, 2007) have been widely used to capture local knowledge, AKT5 offers a unique advantage in integrating both qualitative narratives and quantitative data, making it well-suited for facilitating the representation of local knowledge in a form that allows for systematic analysis and integration with scientific knowledge, thus contributing towards interdisciplinary agroecological research (Sutherland, 2012).

Furthermore, AKT5 is particularly suited for agroecological research as it enables the identification of knowledge hierarchies, causal relationships, and farmers’ decision-making processes regarding land use, crop diversity, and food security (Sinclair and Walker, 1999). Its capacity to capture knowledge heterogeneity across different land-use contexts and social groups made it an ideal choice for our study, which sought to document and co-create knowledge with smallholder farmers in Western Rwanda. While newer methodologies exist, many lack the specificity required for organizing and analyzing local agroecological knowledge in a structured manner. Moreover, the adaptability of AKT5 allows for its refinement and modification in response to contemporary research needs, as demonstrated in recent applications to agroforestry and participatory action research (Coe et al., 2014; Sinclair et al., 2019). Thus, our use of AKT5 is justified by its proven effectiveness, methodological rigor, and adaptability to current agroecological challenges.

This study, which was conducted between August and November, 2015, systematic knowledge-based systems approach (AKT5) (Sinclair and Walker, 1998; Walker and Sinclair, 1998). This involved semi-structured interviews with a stratified sample of 150 willing and knowledgeable informants. The knowledge was then recorded and represented using the AKT5 software (Dixon et al., 2001). The AKT5 local knowledge methodology entails four

stages. All the four stages of the elicitation were applied across all three landscapes namely the degraded, recovering and restored landscapes.

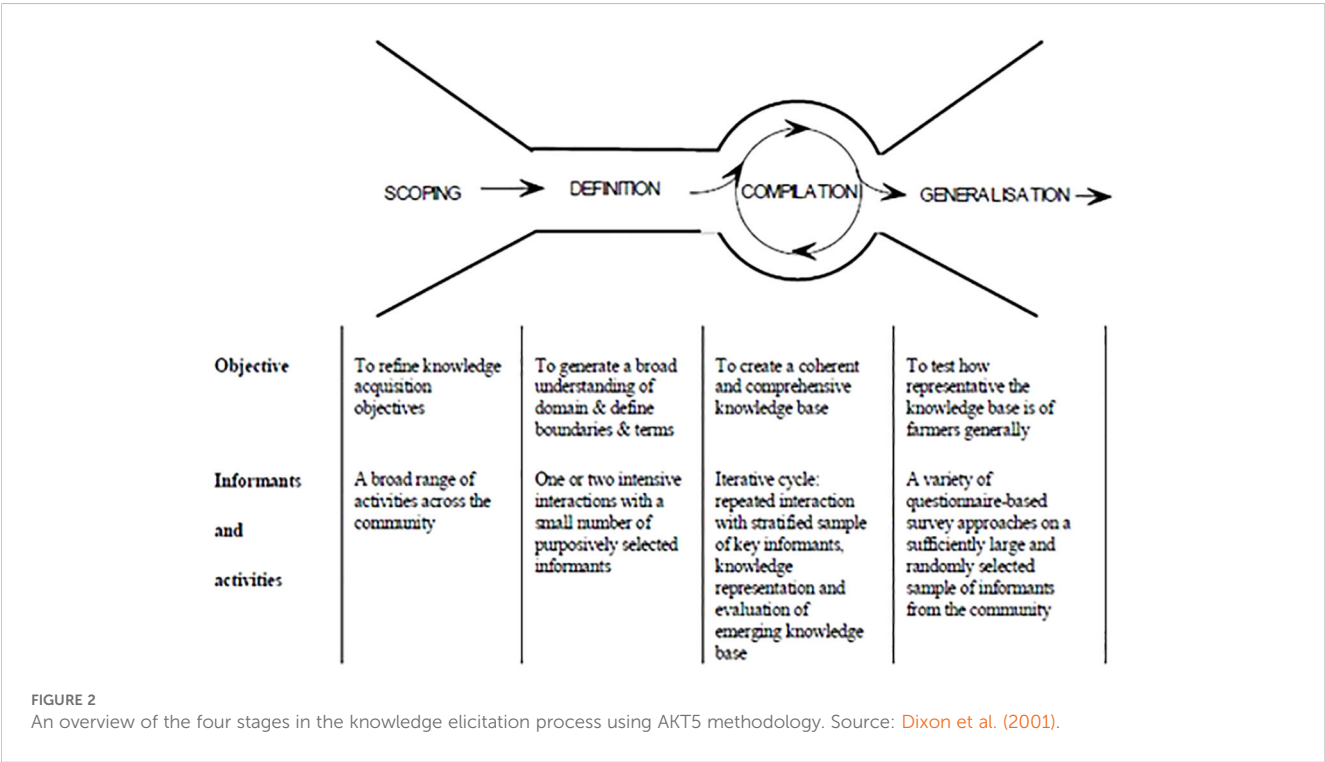
The first (scoping) stage of the AKT5 methodology served to establish mutual familiarity between the researcher and the community, creating a foundation for effective knowledge exchange (Figure 2). The scoping stage activities included participatory transect walks to understand and characterize the landscape biophysical, including farm typologies, community resources, annual and perennial crops grown, and degradation hotspots. These factors were then used as variables for stratifying informants, ensuring a more representative understanding of local knowledge systems. Further, this stage allowed for refining research objectives by clarifying the problem and ensuring the knowledge base aligned with the community's needs and local context. The scoping stage also involved elicitation of non-farmer local informants, which was done through Key Informant Interviews with crop-production experts, agricultural extension officers and local administration. Further, six focus group discussions were held 69 farmers from the three landscapes. While having broad discussion about locally relevant ecosystem services, farmers named food provisioning as their top-most priority, hence the focus of this study. From the discussions, it was noted that crop diversity was low, which informed the need to assess the relationship between crops and food security. Seasons cropping calendars (Yang et al., 2019) were also used to elicit information on the periods that crops are available for consumption and identify food shortage months. This was combined with in-depth discussions on the drivers of food insecurity.

The second (definition) stage of the AKT5 methodology focused on establishing a comprehensive understanding of the

subject domain by setting clear boundaries, identifying key terminologies, and developing a structured framework. To achieve this, key informants were deliberately selected from the community based on their interest, articulateness, depth of knowledge, and willingness to participate, rather than through random sampling. This consisted of six farmers from each of the three landscapes who were randomly selected for in-depth interviews and probing further on the current food security status.

The third (compilation) stage of the AKT5 methodology focused on systematically documenting detailed knowledge within the framework established during the definition stage while capturing variations in knowledge across the community. Rather than seeking statistically representative samples, this stage prioritized in-depth discussions with a small number of highly knowledgeable individuals. The compilation stage involved an iterative process whereby knowledge elicited from individual farmers was re-evaluated through repeated visits to the same farmers to probe further to get additional information or clarifications; which were then recorded and entered into the AKT5 tool. This process was repeated (at least two visits per farmer) until no new information was obtained from each of the respondents. The repeated interviews with the same informants was crucial for gaining deeper explanatory insights and resolving inconsistencies, making willingness to participate, a key selection criterion. A stratified random sampling approach was used to ensure diverse perspectives on the subject matter. Stratification considered key factors such as gender, location of farms along the slope gradient and age, as these were hypothesized to influence knowledge distribution.

The fourth and last stage, which is referred to as the generalization stage of the AKT5 methodology aimed to assess



the representativeness of the knowledge-base obtained from a small group of informants by testing its validity across the broader community. This required a statistically representative random sample, typically consisting of at least 100 individuals who were not previously interviewed. The generalization stage involved formulating key crop diversification – food security research questions based on issues deemed context-relevant based on the in-depth knowledge obtained during the previous three stages. Pre-testing of the questionnaire was then conducted with 12 farmers (four from each of the three landscapes). Once the questionnaire was refined, it was then administered to 150 farmers (50 farmers from each of the three landscapes). The 150 farmers were interviewed for both 1995 and 2015 food security status. Willing farmers were then selected through longitudinal and horizontal transects. The sample comprised of 83 men and 67 women. Results presented here were generated at the last (generalization) stage of AKT5 local knowledge elicitation. The key objectives of this stage included validating the knowledge base to ensure it accurately reflected the community's collective understanding. Additionally, this stage examined how knowledge was distributed among different community members and identified variations in perspectives. It also provided an opportunity to supplement the existing knowledge base with additional details that may have been overlooked during the compilation stage, thereby refining and enhancing the overall understanding of the domain.

The AKT5 methodology is therefore designed to facilitate the systematic elicitation and organization of local knowledge in a way that integrates both qualitative insights and structured analysis (Dixon et al., 2001). By employing a multi-stage approach, AKT5 allowed for an iterative refinement of research questions, ensuring that the final data collection phase captures the most relevant and context-specific knowledge (Walker and Sinclair, 1998). As discussed in the above stages, the initial stages thus involved participatory knowledge elicitation with farmers, experts, and local stakeholders, which helped structure the knowledge base before conducting large-scale surveys (Altieri et al., 2015). While the final stage consisted of individual interviews, it built upon the socially embedded knowledge networks identified earlier, allowing for both individual and collective knowledge processes to be considered. This methodological approach ensured that the study captured the complexity of local knowledge systems, while providing a structured means for comparison with scientific knowledge (Dixon et al., 2001).

Over the years, the AKT5 methodology has however evolved to enhance its applicability in complex agroecological and food system research. Initially designed to systematically structure and analyze indigenous ecological knowledge (Dixon et al., 2001; Walker and Sinclair, 1998), its refinement has integrated participatory validation processes, gendered knowledge systems, and multi-scalar assessments. In our current research revisiting agroecological transitions in Western Rwanda, AKT5 was adapted to capture longitudinal changes in local knowledge across different land degradation contexts. By incorporating a Paired Catchment Assessment and integrating recent literature, this study strengthens AKT5's ability to contextualize crop diversity–food security–land

degradation dynamics within evolving policy and environmental challenges (Kuria, 2019). This refinement underscores the importance of local knowledge in shaping adaptive, context-specific, and inclusive food policies, ensuring that agroecological transitions align with diverse socio-ecological realities.

2.3 Data analysis methods

AKT5 tool was used to analyze and qualitatively interpret data and knowledge elicited through the first three stages of the AKT process explained earlier (Sinclair and Walker, 1998; Walker and Sinclair, 1998). It involved breaking down knowledge into unitary statements which were then represented using formal grammar and local taxonomies where applicable. While local taxonomies and qualitative statements captured the depth and context of indigenous knowledge, the process of converting these into variables allowed for comparative analysis and pattern recognition across different knowledge holders and contexts. In this study, the transformation of qualitative statements into variables was conducted with careful consideration of preserving meaning while enabling broader synthesis. This formed a basis for formulating the questionnaire for collecting quantitative data. The Generalization stage data was recorded in Microsoft Excel and was then exported to R statistical software (R Development Core Team, 2013) for further analysis. Frequency statistics (including percentages) were run to show the number of farmers that held knowledge about a specific food security aspect. Results were also presented using bar plots generated using the 'ggplot' function. Due to the categorical nature of the variables, where a stratum had a sample size of at least five, a Chi-square Test of Independence was applied to examine associations and variations in knowledge distribution among different participant groups and determine whether the sample data was consistent with the distribution that had been hypothesized (Mchugh, 2013). This step aligns with the mixed-methods approach, integrating qualitative insights with quantitative validation to strengthen the reliability of findings.

3 Results

3.1 Decreasing on-farm crop diversity trends between 1995 and 2015

We sought to understand whether on-farm crop diversity has changed or remained the same between 1995 (before genocide period) and 2015 (when this study was undertaken). We requested all farmers interviewed to name the food crops they were growing in 2015 and in 1995. Results from the 150 farmers interviewed in Gishwati indicated a notable decrease in the number of farmers growing some of the annual crops or complete disappearance of some annual crops from farms between 1995 and 2015; and inversely an increase in the number of farmers growing perennial crops in 2015 compared to 1995.

A total of 10 annual crops were grown by farmers between 1995 and 2015 (Figure 3). In both years, the main annual crops grown consistently by majority of farmers were beans (94% and 98%) and Irish potatoes (77% and 86%) respectively. However, there were significant differences ($p=0.001$) in the number of farmers growing sorghum, peas and maize between the two years. In 2015, no farmer was growing sorghum, which was being grown by 68% of farmers in 1995; while only 1% of farmers grew peas, which was being grown by over 50% of farmers in 1995. Maize too was being grown by fewer farmers (35%) in 2015 compared to 1995 (83%). However, no farmer reported growing amaranth in 1995 but it was being grown in 2015 by 15% of farmers.

Seven perennial crops were being grown between 1995 and 2015 (Figure 4). There was an increase in the number of farmers growing avocados and tree tomatoes in 2015 compared to 1995, though the differences were not significant. Avocados were being grown by at least 57% of farmers in 2015 compared to 45% in 1995. The number of farmers growing bananas decreased significantly ($p=0.05$) between 1995 and 2015 while guavas disappeared by

2015. Unlike in 1995, in 2015, farmers were growing cassava (*Manihot glaziovii*), whose leaves played a key role in the nutritional diets of farmers as vegetables ('isombe' in kinyarwanda).

The number of farmers growing some of the annual and perennial crops varied with land degradation status. For annual crops, in both 1995 and 2015, sweet potatoes were mostly grown in the Degraded landscape, while Irish potatoes were mostly grown in the Recovering and Restored landscapes. In 1995, sorghum was mostly grown by farmers in the Recovering and Restored landscapes, while in 2015, maize was mostly being grown in the Restored and Recovering landscapes. However, there was no significant difference in number of farmers growing beans in both years across the three landscapes.

For perennial crops, in both 1995 and 2015, bananas were mostly grown in the Degraded landscape. In both 1995 and 2015, a higher proportion of farmers in the Degraded landscape grew avocados compared to other landscapes. In the Recovering and Restored landscapes in 2015, there was increased growing of tree tomatoes, which was mainly due to

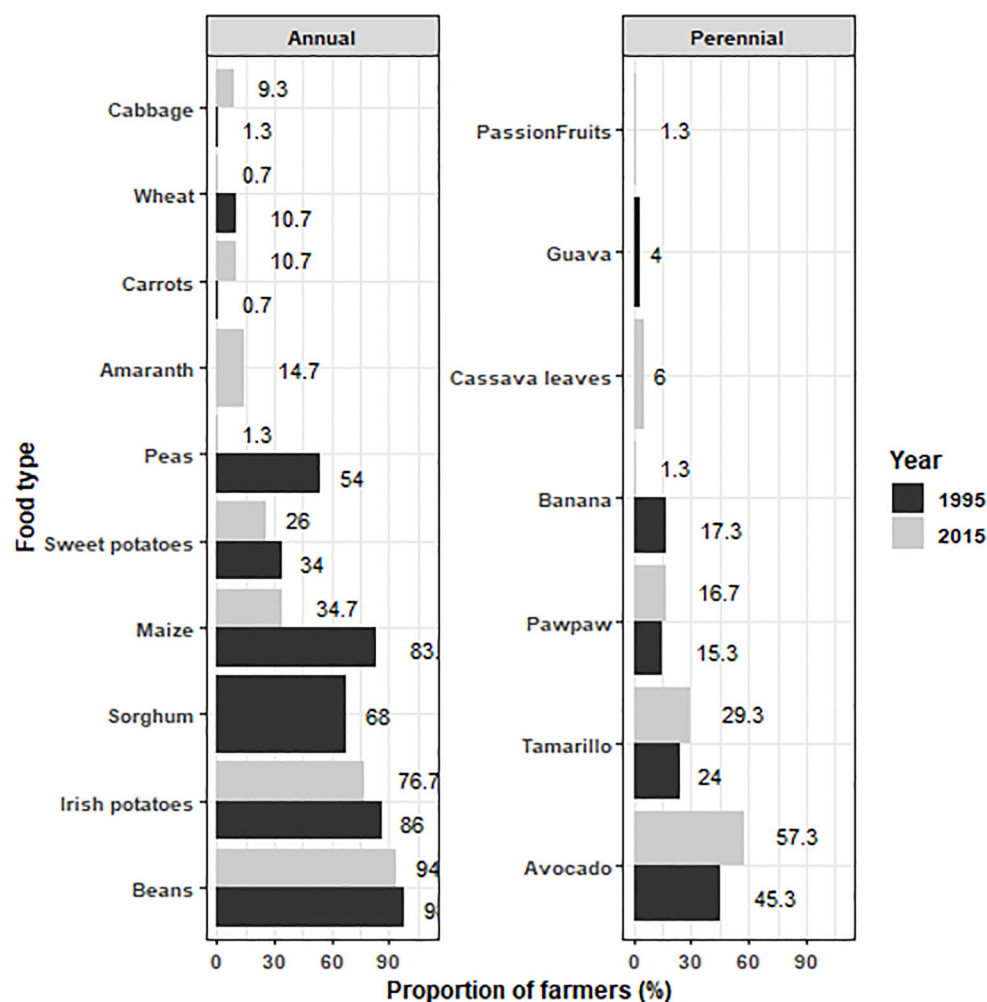


FIGURE 3
Proportion (%) of farmers growing crops in 1995 and 2015.

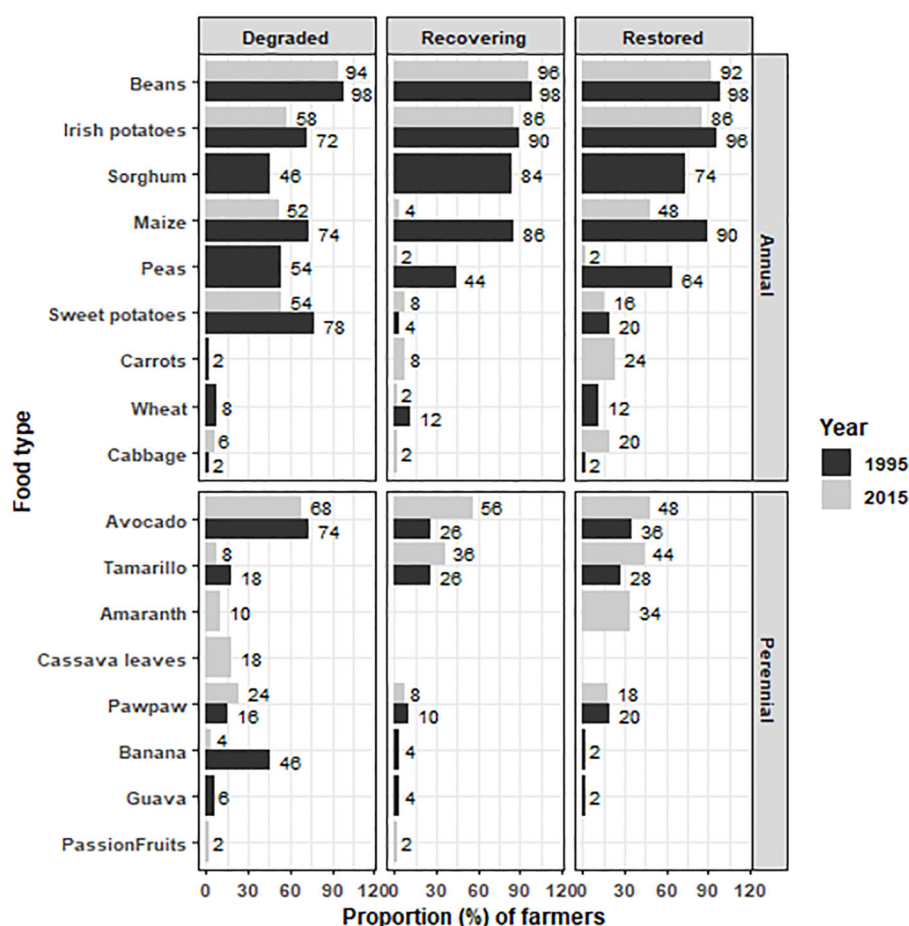


FIGURE 4
Proportion (%) of farmers growing crops in 1995 and 2015 by degradation level.

distribution of quality germplasm by projects such as the Trees for Food Security project through the World Agroforestry Centre (ICRAF).

3.2 Farmers' knowledge of drivers influencing crop diversity

Farmers identified six drivers influencing annual crop diversity, which occurred across four scales (regional, national, landscape and farm level) and of which four drivers varied significantly with land degradation status. The drivers were: at the national level (policies on crop intensification and eviction of farmers from Gishwati encroachment), at the regional level (climate change), farm level (land shortage and abandonment of slow maturing crops); and at the landscape scale (crop diseases).

According to majority of farmers (76%), the main driver that contributed to the decrease in annual crop diversity between 1995 and 2015 was the introduction of the Land-use Consolidation and Crop Intensification Program (CIP) that was launched in September 2007 as a policy by the Government of Rwanda. The program aimed at promoting the cultivation of three high value

crops namely Irish potatoes, beans and maize, which fetched high income which the government believed would improve farmers' livelihoods significantly. Farmers however felt that specialization of a few high value crops has led them to abandon other crops they were growing, which were viewed as 'low value', thus resulting in decreasing diversity of such crops across farms. There were significant differences ($p=0.05$) in the number of farmers who mentioned CIP program by degradation status, with the driver being mostly mentioned by a significantly higher number of farmers in the Restored landscapes (88%) and Recovering landscapes (78%), compared to Degraded landscape (62%) (Figure 5).

Land shortage was the second most frequently mentioned driver of decreasing annual crop diversity (55% of farmers of all farmers). This was mainly blamed on the natural population increase among households, that led to sub-division of land amongst the kin. There were significant differences ($p=0.001$) in the number of farmers who mentioned land shortage, with fewer farmers in the recovering landscape mentioning this driver, significantly different from the other landscapes. Thirdly, 49% farmers reported having gradually abandoned slow growing and maturing crops such as sorghum and banana for fast-growing crops such as maize and Irish potatoes. There were significant differences ($p=0.05$) in the number of farmers

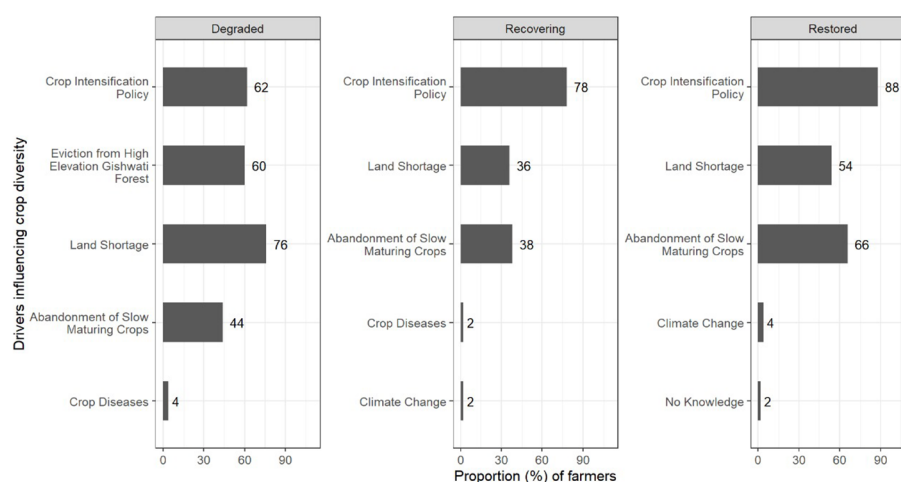


FIGURE 5
Drivers influencing annual crop diversity between 1995 and 2015 by degradation level.

who mentioned this driver, with it being mostly mentioned in the Restored landscape (66%) compared to degraded landscape (44%) and recovering landscape (38%).

The fourth driver, which was only reported by farmers in the degraded landscape by 60% of farmers (significant at $p=0.001$) was the eviction of farmers from Gishwati forest as the landscape is directly adjacent to the protected forest. When farmers were evicted from Gishwati forest which sits at a high elevation of above 2400 meters above sea level (m.a.s.l.) where they were cultivating crops such as wheat and peas that do well in high elevation, they abandoned growing such crops when they were relocated to the low-lying areas of below 2000 m.a.s.l. which are unfavorable for growing such crops. Crop diseases and climate change drivers were mentioned negligibly by farmers across all landscapes.

Farmers identified two main drivers affecting perennial crop diversity, namely the increase in availability of tree seedlings (66%); and training of farmers on tree management practices, especially propagation methods including grafting of fruits such as avocados (34%). In the Recovering and Restored landscapes, there was increased in the number of farmers growing of tree tomatoes, which was mainly due to distribution of training and distribution of high-quality germplasm attributed to interventions such as by the Trees for Food Security project.

3.3 Relationship between crop diversity and food security

A total of 83% of farmers reported being food insecure, 96% and 86% of farmers from the degraded and restored landscapes respectively perceived themselves as being food insecure, significantly different ($p=0.05$); compared to 68% of farmers from the recovering landscape. Farmers identified four local indicators they use to assess their food insecurity status namely food shortage during certain months of the year, taking fewer meals per day throughout the year, consuming less preferred food and reducing food portions per meal.

The main indicator farmers use to assess whether they are food insecure as mentioned by 51% of farmers was food shortage during certain months of the year (mainly July to November), attributed to the depletion of food reserves during this five-month period when the three major crops (maize, Irish potatoes, beans) which farmers highly depend on are growing and not yet mature for consumption (Table 1). These dominant annual crops (beans, Irish potatoes, maize) are harvested and available for consumption only between December to February/March and from June to August. Due to a slightly different cropping calendar and variation of some food types grown, food-insecure months in the Degraded landscapes were from March to May and August to November while in the Recovering and Restored landscapes were from March to June and September to November. Perennial crops mainly tree crops such as avocados and tree tomatoes and cassava leaves were mostly available from June to February, and farmers reported relying on them to fill the food gap during the period when annual crops were not available.

The second overall most frequently mentioned indicator of food insecurity was farmers resulting to taking fewer meals per day throughout the year (47%). Farmers and their dependents resulted to taking one or two meals (most important meals) instead of the usual three throughout the year, without reducing food serving proportions per meal. According to farmers, the most important meal is dinner, followed by breakfast and lastly lunch. This coping strategy ensured that food reserves were utilized sparingly to last longer.

The third most frequently mentioned indicator (22%) was when farmers resulted to consuming less preferred foods such as sweet potatoes, cassava leaves and bananas, when the preferred foods such as Irish potatoes, beans and maize were not available. The fourth indicator was reducing food portions per meal (15%). This was achieved through taking three meals in a day but reducing serving portions to ensure little food is consumed.

There were significant differences in the number of farmers mentioning all indicators of food insecurity by land degradation status (Figure 6). Reducing food portions per meal was mainly

TABLE 1 Annual and perennial food crop availability calendar.

	Botanical Name	Food Type	Main Rainy Season			Dry Season			Lighter Rainy Season			Dry Season		
			Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb
Degraded Landscape														
Annual Crops	<i>Phaseolus vulgaris</i> L.	Beans												
	<i>Solanum tuberosum</i> L.	Irish potatoes												
	<i>Zea mays</i> L.	Maize												
	<i>Brassica oleracea</i> var. <i>capitata</i>	Cabbage												
	<i>Daucus carota</i> subsp. <i>Sativus</i>	Carrots												
	<i>Amaranthus</i> spp.	Amaranth												
	<i>Ipomoea batatas</i>	Sweet potatoes												
Perennial Crops	<i>Carica papaya</i>	Pawpaw												
	<i>Musa</i> spp.	Banana												
	<i>Cyphomandra betacea</i>	Tamarillo												
	<i>Psidium guajava</i>	Guava												
	<i>Persea americana</i>	Avocadoes												
	<i>Passiflora edulis</i>	Passion fruits												
	<i>Manihot glaziovii</i>	Cassava leaves												
Recovering and Restored Landscapes														
Annual Crops	<i>Solanum tuberosum</i> L.	Irish potatoes												
	<i>Zea mays</i> L.	Maize												
	<i>Pisum sativum</i>	Peas												
	<i>Brassica oleracea</i> var. <i>capitata</i>	Cabbage												
	<i>Daucus carota</i> subsp. <i>Sativus</i>	Carrots												
	<i>Phaseolus vulgaris</i> L.	Beans												
	<i>Amaranthus</i> spp.	Amaranth												
	<i>Triticum aestivum</i>	Wheat												
	<i>Ipomoea batatas</i>	Sweet potatoes												
Perennial Crops	<i>Psidium guajava</i>	Guava												
	<i>Carica papaya</i>	Pawpaw												
	<i>Cyphomandra betacea</i>	Tamarillo												

(Continued)

TABLE 1 Continued

	Botanical Name	Food Type	Main Rainy Season			Dry Season			Lighter Rainy Season			Dry Season		
			Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb
Recovering and Restored Landscapes														
	<i>Persea americana</i>	Avocadoes												
	<i>Passiflora edulis</i>	Passion fruits												

Different colors are used to distinguish between annual and perennial crops.

mentioned in the Degraded landscape, varying significantly ($p=0.001$) from other landscapes. The other three indicators varied significantly among landscapes ($p=0.05$); with the main indicator mentioned in the Recovering landscape being food shortage during certain months (64%) and taking fewer meals per day throughout the year (62%); while consuming less preferred food was mostly mentioned in the Restored landscape (34%). On the other hand, in the Degraded landscape, all four indicators were mentioned by almost similar proportions of farmers.

3.4 On-farm and off-farm food sourcing trends between 1995 and 2015

We also sought to understand whether over time, farmers have become increasingly dependent on off-farm compared to on-farm food sourcing to meet their food needs. Farmers reported that due to decreased crop diversity discussed in earlier sections which led to them experiencing food insecurity, they had resulted to outsourcing food from off-farm sources, mainly buying from the market. As illustrated in Table 2, majority of farmers had become more

dependent off-farm sources such as on the market, with food produced on-farm supporting farmers for average 6.6 months annually in 2015 compared to 10.1 months in 1995.

In 1995, more farmers from the recovering landscape relied more on on-farm and less on off-farm food sources in both year periods. Conversely, more farmers from the degraded landscape relied more on off-farm and less on on-farm food sources in both year periods. In 2015, there were variations, though not significantly different, in on-farm and off-farm food sourcing along a land degradation gradient, with farmers in the Recovering landscapes depending on their farms slightly more (7.8 months) in 2015 compared to the Restored (6.3 months) and Degraded landscape (5.7 months).

Figure 7 shows that in 2015, majority of farmers outsourced from the market and consumed eight out of the nine annual food crops they grew on their farms to supplement the food demand and outsourced 11 perennial crops though they only grew six. For annual crops, apart from beans that were grown by majority of farmers, farmers depended on off-farm sources for majority of other foods they consumed, significantly differing from on-farm sources. The food sourcing (growing and consumption) differences were especially apparent in the recovering landscape.

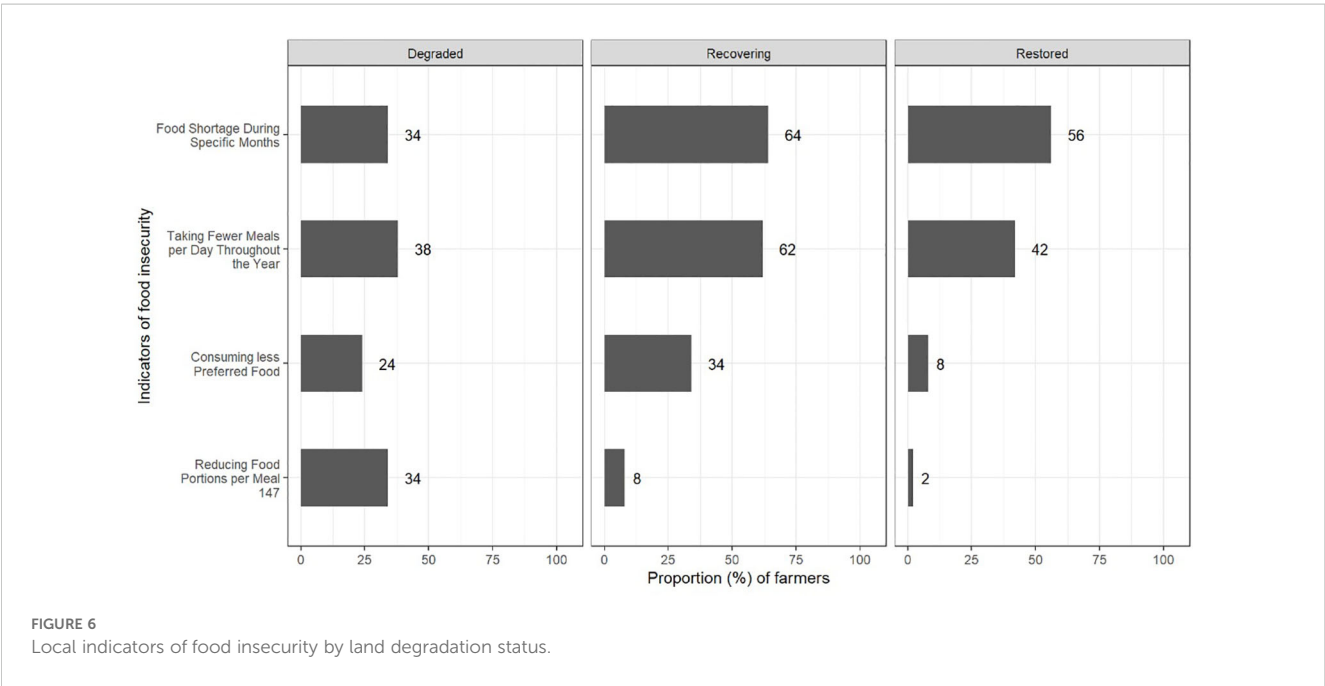


TABLE 2 Comparison of 1995 and 2015 food sourcing proportion (months per year).

Food source	On-farm		Buy from market		Buy from neighbors		Borrow from relatives	
	1995	2015	1995	2015	1995	2015	1995	2015
No. of months								
All landscapes	10.1	6.6	1.5	5.4	0.1	0	0	0.01
Degraded	8.8	5.7	2.4	6.2	0.2	0	0	0.04
Recovering	11.4	7.8	0.6	4.2	0	0	0	0
Restored	9.9	6.3	1.5	5.7	0	0	0	0

4 Discussion

4.1 The role of local knowledge in promoting agroecological principles towards sustainable food systems

This study aims to revisit and validate findings of local knowledge data collected in 2015 in line with current literature to assess and understand changes, trends, and developments over time; and will provide continuity in understanding long-term intervention impacts of interventions. Findings from the current

local knowledge study has brought out in depth understanding of seven out of the 13 agroecological principles that should guide food systems towards transitioning to becoming sustainable towards achieving sustainable food systems through enhanced crop diversity. The following subsections discuss each of the seven agroecological principles emerging from the results presented, which fall under two of the three operational principles on sustainable food systems (HLPE, 2019). Four principles fall under the strengthening resilience operational category namely: soil health, biodiversity, synergy and economic diversification; while three fall under secure social equity namely: social values and diets,

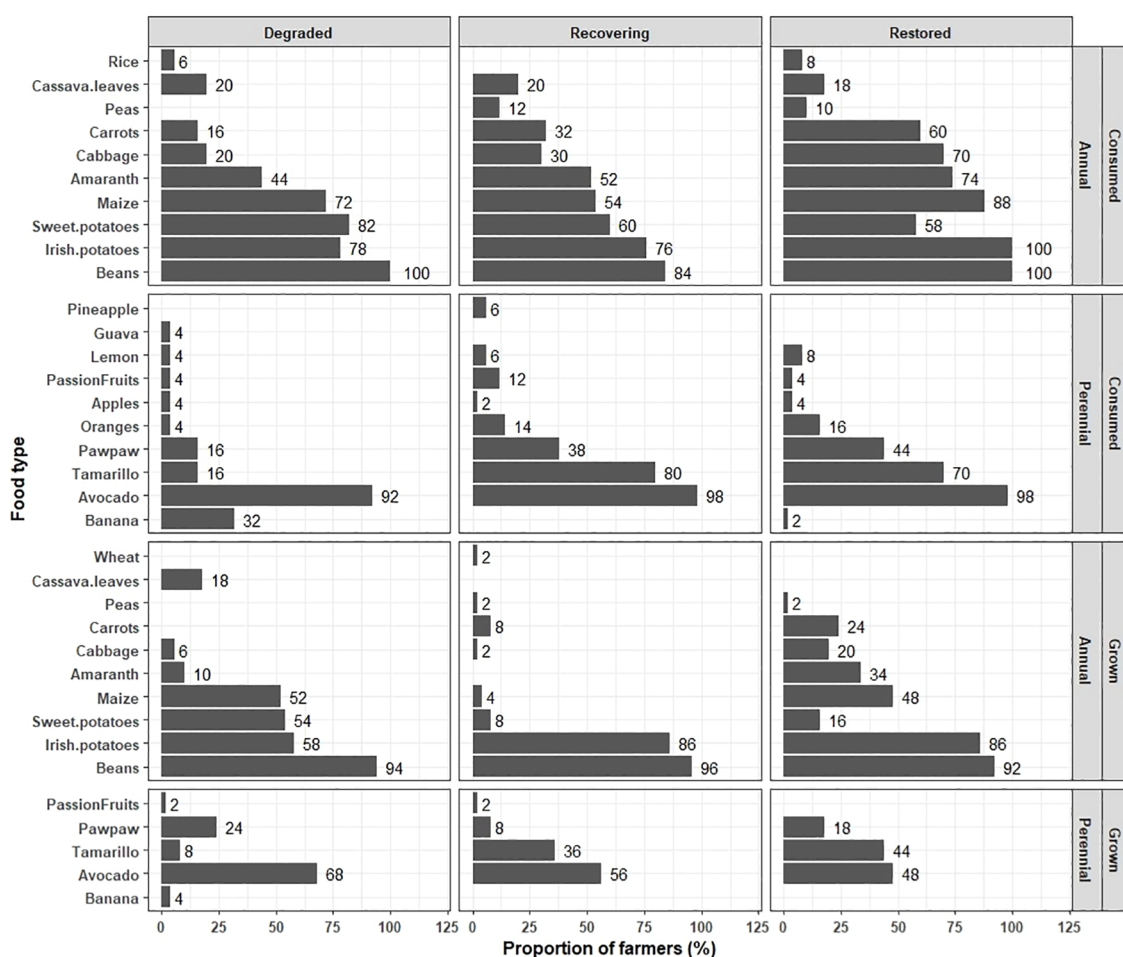


FIGURE 7
Proportion (%) of farmers who sourced food on-farm and off-farm in 2015.

co-creation of knowledge and participation. However, no agroecological principle was reported in relation to the role of crop diversity in improving resource efficiency, contrary to other studies that have highlighted this as a critical role (Chittapur, 2017; Isbell et al., 2017).

4.1.1 Soil health

Results showed significant differences in farmer's knowledge of various food security aspects namely crop diversity, food availability trends; drivers influencing food crop diversity and indicators of food insecurity across the three landscapes sampled along a land degradation gradient (degraded, recovering and restored systems). For example, results indicated lower percentage of farmers growing crops that have higher nutrient requirements (fertile soils) such as Irish potatoes and maize in the degraded landscapes. In a previous study in the same landscapes, land degradation was found to influence soil quality as soils in the degraded landscape were found to have lower organic matter and lower diversity of beneficial macrofauna species hence less productive compared to recovering and restored landscapes (Kuria et al., 2019). Studies have shown that crops that have higher nutrient requirements are often not adapted to low-input systems and can only be grown successfully in degraded and less fertile land through involving a high-input farming system that relies heavily on external inputs such as fertilizers (Bucagu et al., 2020; Mugendi, 2013). Heavy reliance on external inputs further leads to decreased soil health and quality through pollution (Singh et al., 2023). Results further indicate that land shortage was the main driver of low crop diversity in the degraded landscape as mentioned by 76% of farmers. Studies show that increased population leads to land fragmentation and decreased average household land sizes. This results into adoption of intensified farming practices such as continuous cultivation without fallows; and specializing on high income monocrops in order to maximize on returns on land (Jiang et al., 2021). This in return has negative effects on soil health due to soil fertility depletion.

These results demonstrate the need for Rwandan government and other food policy actors to adopt agroecological practices that promote integrated soil management practices including structural practices that control soil erosion, biological and cultural practices (Garritty et al., 2010; Mutemi et al., 2017); including practices that restore soil health in the long-term mainly aimed at increasing soil organic matter and the introduction of shrubs and crops that improve soil fertility on the degraded systems such as the nitrogen-fixing leguminous crops (Bolo et al., 2024; Yao et al., 2023). Gradually, once degraded soil is restored, farmers can then be able to diversify their systems through growing crops that have high nutrient intake such as maize and Irish potatoes in such landscapes.

4.1.2 Biodiversity

Results in Figure 3 and Figure 4 show that on farm annual crop diversity decreased between 1995 and 2015, with some crops such as sorghum, peas and wheat disappearing from farms; while only a few crops were prioritized mainly Irish potatoes, beans and maize

blamed on the Crop Intensification Program (76%), land shortage (55%) and abandonment of slow growing crops (49%). Despite the interventions of the crop intensification Program, which was highly heralded as an example of the 'new' Green Revolution (Cioffo et al., 2016) leading to an increased yields for these priority crops, the program has also led to decreasing annual crop diversity (Seburanga, 2013) due to promotion and intensification of only a few crops while other crops viewed as of 'low value' are ignored.

Local knowledge acquisition highlighted the importance of promoting and maintaining biodiversity; and led to the realization of the negative impacts of decreasing annual crop species diversity in space and time (between 1995 and 2015) such as food insecurity during certain months that priority crops were still growing and not ready for consumption. Studies show that gradual specialization in few crops results into the farming systems becoming more simplified and less resilient (Altieri and Nicholls, 2020). This is because monocultures lead to the gradual agricultural biodiversity loss and increase vulnerability of a system to adverse threats such as climatic variabilities, pests and diseases (Barthel et al., 2013; Luedeling et al., 2014).

Further, the specialization on a few exotic perennial crops at the expense of native perennials has been blamed on the loss of on-farm diversity in Rwanda (Ruticumugambi et al., 2024). Still, recent studies which revisited Rwanda's crop intensification program further noted that specialization in the few priority crops overlooks the heterogeneity and dynamic nature of Rwandese farmers' social, economic and environmental context (Franke et al., 2019; Kim et al., 2022). This has resulted in inequalities in benefits generated from the CIP program.

4.1.3 Synergy

Results from the cropping calendar indicated synergies and complementarity brought about by the integration of perennial crops, in this case trees and annual crops in achieving food security all year round. This is because different tree species play unique roles in the system, both through provisioning ecosystem services or ecologically and products mature at different periods of the year (Carsan et al., 2014). For example, having more fruit tree species, whose fruiting phenology is varying means that fruits are available in different months of the year, hence continued access to products and income, which supplement annual food crop sources.

Increasing crop diversity (annual and perennial) is especially critical and beneficial in restoring degraded lands because it not only demonstrate the role that individual crops play towards enhancing food security throughout the year, but enhances the functional diversity roles played by various crops collectively such as nutrient cycling, erosion control, and ecosystem products (Di Falco and Chavas, 2009). For example, farmers in Rwanda reported achieving higher yields of potatoes, maize, and beans on farms with trees in the humid region, and higher yields of beans in the semi-arid regions (Cyamweshi et al., 2023). Further, expanding crop portfolios is viewed as an ecological adaptation to climate change and enhanced resilience from diseases (Meldrum et al., 2018). A recent study in Rwanda (Hashakimana et al., 2023) has further revealed that high carbon sequestration and subsequently high soil

organic carbon was found among mixed-cropping systems compared to the CIP monocropping systems. By elevating the multifunctionality of systems, crop polycultures can achieve greater functional diversity (Finney and Kaye, 2017). Dusingizimana et al. (2024) further notes that dietary diversity in Rwanda in the recent years has been enhanced through integrating livestock within cropping systems. The interaction of components in both space and time results in numerous advantages and synergies for stakeholders across a wide spectrum of products and services. This therefore promotes complementarities through promoting the production of multiple ecological products and services simultaneously (Matsushita et al., 2016).

4.1.4 Economic diversification

While the government of Rwanda introduced CIP with the aim of achieving economic growth, food security and livelihood development (Kim et al., 2022) but which results show led to reduced crop diversity on the contrary. Farm diversification through crop diversification has been found to contribute towards livelihood resilience by enhancing farm productivity by providing additional income and nutritional diversity generated through off-farm sourcing (Makate et al., 2016; Nsabimana et al., 2021). In addition, including different crops in a farming system acts as a type of natural insurance against unfavorable markets, drought; pests and diseases (Benin et al., 2004). Hence farmers can still benefit from and rely on some crops when other crops in their systems fail. On the other hand, specialization in a few crops by the same population has been reported to cause low economic returns due to market competition (Byerlee et al., 2014). Miklyaev et al. (2021) calls for the need for Rwanda government to respond to market demands while designing future crop intensification programs. Further, having different annual and perennial crops maturing at different times of the year leads to diversified income streams as farmers can sell their farm produce throughout. (Niether et al., 2020) found the total system yields for mixed agroforestry systems to be ten times higher than monocrops, contributing to food security and diversified income.

4.1.5 Co-creation of knowledge

Results of this study demonstrated that smallholder farmers have detailed and explanatory knowledge about crop diversity and the role it plays towards meeting their food security and livelihood needs. They were able to describe drivers that have influenced their annual and perennial crop diversity, cropping calendars including the role perennial crops play in their agricultural systems; and indicators of food insecurity. Interviewing farmers across different land degradation status further brought about heterogeneity of context. Such knowledge would be critical in complementing the already available scientific knowledge of the area through providing in-depth understanding about the complexity and heterogeneity of the Western Rwanda agroecological systems (Sinclair et al., 2019; Wezel et al., 2020); and hence would guide food policy makers to customize interventions to the context (Rossing et al., 2021).

Local knowledge itself falls under the co-creation of knowledge agroecological principle and plays a key role in the development of

locally adapted practices; and was the over-arching agroecological principle guiding this study. Local knowledge is inherently context-specific, shaped by socio-ecological interactions and passed through generations (Berkes, 2009). Unlike scientific knowledge, which often seeks universal principles, local knowledge is adaptive and dynamic, making its validation a complex process that extends beyond mere comparison with scientific findings (Agrawal, 1995). Our study applied a co-creation approach that integrates scientific and local knowledge through an iterative process of elicitation, interpretation, and validation with farmers (Chambers, 2007; Fuchs et al., 2023; Kuria et al., 2024). This approach aligns with growing recognition that knowledge pluralism, where multiple ways of knowing are equally valued enhances agricultural innovation and policy relevance (Turnhout et al., 2019).

Due to the heterogeneity of smallholder farming systems, policy makers should ensure that they design food security policies informed by the local context (Coe et al., 2014). This should begin with gaining local understanding and knowledge of which measures are appropriate in each context including not only direct measures such as structural changes but indirect policy measures such as improving agricultural infrastructure, understanding the biophysical and socioeconomic, and providing farmers with new farm technologies (Berazneva and Lee, 2013). Also of importance is adapting food programs to dynamic local indicators such as climate change, soil conditions and land degradation (Kuria et al., 2019, 2023) and where adaptation information is unavailable, policy makers should communicate such information to local communities (Thornton et al., 2018).

Agroecology is based on bottom-up and territorial processes, helping to deliver contextualized solutions to local problems and hence it depends on local contexts, constraints and opportunities. This calls for the need to adapt food systems to the current context and viewing farmers as co-innovators of knowledge rather than passive adopters of technologies. It is important to collectively find innovative ways of increasing the transformational resilience and adaptive capabilities of smallholder farmers (Savage et al., 2020). This will result into co-learning and co-creation of new knowledge (Frias-Navarro and Montoya-Restrepo, 2020; Marinus et al., 2021). There is therefore urgent need to rethink and formulate food policies that incorporate local food systems rather than that are top-down and not informed by what works locally (Galimberti et al., 2020).

The findings of this study contribute to the growing discourse on local knowledge and knowledge co-creation in agroecological transitions, aligning with and extending previous research. Similar to Tolinggi et al. (2023), who explored knowledge transfer across generations, this study revealed that farmers in Gishwati rely on intergenerational knowledge to navigate the crop diversity–food security–land degradation nexus. However, while Tolinggi et al. (2023) emphasize how traditional farming wisdom is passed down, the current study highlights the disruptions caused by external policies, such as Rwanda's Crop Intensification Program, which has influenced knowledge retention and adaptation processes. Moreover, Arifah et al. (2023) examined knowledge co-creation in response to climate change, emphasizing the importance of integrating scientific and local knowledge for adaptive decision-

making. Our findings similarly underscore the role of farmers' experiential knowledge in shaping agroecological practices, particularly in relation to crop diversity and resilience strategies. However, while Arifah et al. (2023) focus on farmer–scientist collaboration, this study revealed a gap in structured co-creation mechanisms, with farmers primarily relying on informal knowledge networks rather than institutionalized participatory platforms.

Additionally, Arham et al. (2024) investigated knowledge construction among coffee farmers, highlighting the role of collective learning in improving productivity and sustainability. Our study complements this perspective by demonstrating how knowledge co-creation extends beyond productivity concerns to encompass broader agroecological principles, such as biodiversity conservation and food security. While both studies emphasize the significance of shared learning, our findings suggest that knowledge fragmentation due to shifting policy priorities can hinder the continuity of local knowledge systems.

Furthermore, local knowledge systems are shaped by ecological, socio-economic, and gendered factors, influencing the adoption of agroecological practices. Women and men contribute distinct expertise, women often manage seed selection and intercropping for resilience, while men focus on land preparation and cash crops (Bezner Kerr et al., 2019; Meinzen-Dick et al., 2019). Recognizing these gendered roles is essential for developing sustainable, locally adapted solutions (Ramirez-Santos et al., 2023). Policies that overlook gendered knowledge risk reinforcing inequalities. Inclusive, participatory approaches are crucial for co-creating knowledge and designing equitable contexts (Nyantakyi-Frimpong et al., 2017).

4.1.6 Social values and diets

Results indicate that 83% of farmers reported being food insecure. Results from the seasonal calendar presented in Table 1 indicated that households that had higher crop diversity including perennials such as fruits were more food secure, especially during food gaps when annual crops are unavailable. This was the main indicators of food insecurity reported by farmers whereby July to November were named as the most food insecure months. Seasonal food shortage has been reported to result to poor maternal and child health due to hunger and deprivation of micronutrients critical for growth (Belayneh et al., 2020; Fraval et al., 2020; Waswa et al., 2021). Adjimoti and Kwadzo (2018) further observes that increased crop diversity in Benin ensured that different crops are available for consumption throughout the year, hence fulfilling the accessibility pillar of food security. This was also echoed in Rwanda by (Ndoli et al., 2021), where on-farm trees were found to act as a safety net for many smallholder households, with food insecure households relying more on income from sale of trees to meet their food needs. Studies indicate a positive co-relation between tree cover and dietary diversity because of availability of fruits and vegetables provided by trees (Ickowitz et al., 2014; McMullin et al., 2019). Agroforestry trees provide nutrient-rich foods that contribute

towards improved dietary diversity of women and children (Lourme-Ruiz et al., 2021).

Taking fewer meals per day throughout the year, consuming less preferred foods and reducing food portions per meal were also reported as indicators of food insecurity (Figure 6). Decreasing crop diversity also results into nutritional insecurity as households who traditionally enjoyed a wide diversity of nutritious crops become confined to consuming foods only a few food crops throughout the year, which may have low nutritional and dietary value hence may lead to poor health (Burchi and De Muro, 2016). Low dietary diversity, malnutrition and micronutrient deficiencies have been widely reported among Rwandese women and children (Sly et al., 2023; Xavier et al., 2024). Consuming less preferred food was also reported elsewhere in Peru and Ethiopia (Ambikapathi et al., 2018; Dessalegn, 2018). Globally, low crop diversity has been linked to reduced nutritional stability, as it often results in a focus on crops with fewer nutrients or nutrients already abundant in the existing food system (Nicholson et al., 2021). These findings go on to show that food insecurity manifests in different ways in different context, and communities cope in different ways, hence the need to develop food policies and programs that are informed by the different food insecurity indicators.

The abandonment of slow maturing crops such as sorghum and bananas was also reported as a driver of decreasing crop diversity. This has not been widely reported in literature. In Rwanda, decreased crop diversity especially loss of indigenous crops has instead been attributed to cultural heritage erosion and disintegration due to colonization and introduction of alien crops (Seburanga, 2013). Rwibasira (2016) further notes that promoting high-value crops through CIP in Rwanda, a country where men dominate economic fronts, has led to alienation of women from crop production activities. Such form of skewed intensification has been reported in other countries including in Ethiopia (Shiferaw et al., 2014); and contributes towards gender inequalities in food production systems. Similar patterns have been documented in Mali, aligning with the paradox of Sikasso, where agricultural intensification does not necessarily translate into improved gender equity (Dury and Bocoum, 2012).

4.1.7 Participation

Farmers attributed Crop Intensification Program (CIP), one of the major agricultural reforms initiated in 2007 by the Rwandan government as the main cause of decreasing annual crop diversity. The main goals of the program were to increase agricultural productivity in high-potential food crops (maize, wheat, rice, Irish potato, beans and cassava) and ensuring food security and self-sufficiency across the entire country (Muhinda and Dusengemungu, 2011). Despite the Rwandan government putting in place this food security policy, various authors have noted the lack participation of farmers at the design and operational stages of policies including monitoring of such policies (Namugumya et al., 2020; Welteji et al., 2017). Strengthened collaboration among farmers, local leaders,

extension agents, and agricultural service providers, combined with the practical skills of farmers will significantly enhance participation in the CIP program in the future (Nahayo et al., 2017; Sunday et al., 2024). Using local community's feedback could play a key role in adapting such policies (Moroda et al., 2018). Agroecology represents an approach that is transdisciplinary, participatory, and oriented toward practical action (Méndez et al., 2013; Sinclair et al., 2019). Participation advocates for the involvement of a transdisciplinary team of experts to address the various dimensions of food systems through inclusion of stakeholders and integrating knowledge systems at multiple levels to develop food security innovations that are suited to local context.

Food insecurity and severity is dependent on factors such as gender. For example, in Kenya, Uganda and Tanzania (Silvestri et al., 2015) found that female headed households were more food secure compared to male headed households because women focused on more diverse crops that are not necessarily income oriented compared to men. Participation therefore calls for inclusion whereby all gender are involved due to the unique roles they play in food production, possess unique knowledge, preferences and risk-taking behaviors (Villamor et al., 2014). Sariyev et al. (2021) further observes that participation of all gender leads in inclusive decision making resulting in higher diversity of produced and consumed food.

The link between crop diversity and food security is well-documented, particularly in relation to women's roles in subsistence farming and household nutrition. In the studied landscapes, the shift towards high-value cash crops under the CIP program may have disproportionately affected women's ability to maintain dietary diversity within households. Traditional crops, many of which were rich in essential nutrients, were replaced by market-oriented staple crops, potentially altering household food consumption patterns. While men are involved in high-value, market-oriented crops (Ingabire et al., 2018); women, who are typically responsible for food preparation and household-level food sourcing, likely faced greater challenges in maintaining diverse and balanced diets. Additionally, land shortage and the abandonment of slow-maturing crops both identified as key drivers also had gendered implications. Women often cultivate small, intercropped and diversified plots to ensure food security (Nakazi et al., 2017), but the declining availability of land may have reduced their ability to maintain diverse home gardens.

On-farm perennial crop diversity was found to be increasing between 1995 and 2015, with the main drivers being increased access to quality germplasm of preferred agroforestry tree species and farmers acquiring tree propagation skills. This is mainly attributed to the introduction of participatory approaches (Iiyama et al., 2018; Ndoli et al., 2021) that saw a move from the historical top-down seed and seedling sourcing, to a system where farmers are involved in tree species selection and have access to high quality tree germplasm and are continuously trained on tree propagation and management through ongoing initiatives namely the Trees for Food Security project, which the World Agroforestry Centre was leading at the time this study was undertaken.

4.2 Beyond the farm: implications of off-farm food sourcing on agroecological transitions

Results in Table 2 indicated that over time, farmers have become more dependent on sourcing food from outside their farms, with food produced on-farm supporting farmers for an average of 6.6 months annually in 2015 compared to 10.1 months in 1995. In 2015, farmers in the degraded landscape were more dependent on off-farm food sources (an average of 6.2 months) annually compared to those in a recovering landscape (4.2 months) and a restored landscape (5.7 months). Further, Figure 6 shows that in 2015, majority of farmers outsourced from the market eight out of the nine annual food crops they grew and outsourced 11 perennial crops though they only grew six. This trend is an indication that farmers in Gishwati were often lacking diversity of food crops to sustain their food and nutritional needs. Similar trends of food insecure households relying on off-farm food sourcing such as buying food from the market has been reported (Ali et al., 2014; Fraval et al., 2020).

However, while the Crop Intensification Program (CIP) has played a central role in shaping land use and crop diversity, it is not the sole driver of market dependency and reduced on-farm food provisioning. The increasing monetization of rural economies in sub-Saharan Africa, driven by economic liberalization, globalization, and national policies, has accelerated reliance on off-farm food sources and commercial production. As highlighted in our discussion, this transition aligns with broader trends reported in the literature, where structural shifts in rural economies have contributed to declining crop diversity and heightened food security challenges (Fraval et al., 2019). Recognizing these external pressures is crucial for designing agroecological policies that balance market participation with localized, resilient food systems.

Unlike India's Public Distribution System (PDS), which provides subsidized food grains to vulnerable populations (Kumar, 2021; Pingali et al., 2019), Rwanda's policies have focused on agricultural intensification, particularly through the Crop Intensification Program (CIP), which promoted high-value staple crops but reduced on-farm diversity and increased market dependency (Van de Poel et al., 2014). On-farm food provisioning declined from an average of 10.1 months per year in 1995 to 6.6 months in 2015, with degraded landscapes experiencing the highest reliance on market purchases. While government initiatives like the 'One cow per poor family' 'Girinka' program have improved nutrition and income for some households, they do not offset the vulnerability caused by reduced crop diversity and fluctuating food prices (Fanzo et al., 2020). Additionally, food sourcing strategies varied by landscape degradation status, with farmers in Recovering landscapes maintaining slightly higher on-farm food reliance than those in Degraded landscapes, underscoring the need for targeted interventions to enhance food security in highly degraded areas.

Some studies, however, found that relying on off-farm food sources and income may have a positive effect on food security and nutritional diversity through providing alternative sources of food

(Aboaba et al., 2020; Sibhatu and Qaim, 2018). This is especially so when there are inevitable threats and uncertainties such as extremely poor and unproductive soils, climate change vulnerabilities in areas where populations depend on rain-fed agriculture or due to total crop failure resulting from pests and diseases (Babatunde and Qaim, 2010; Owusu et al., 2011). These findings underscore the potential of combining market-based strategies with on-farm crop diversification to support food security objectives (Morrissey et al., 2024; Ume et al., 2023). However, although this food insecurity coping behavior provides immediate and temporary quick-fix solution, it leads to undesired outcomes in the long run as this behavior takes farmers away from investing in improving their farms (Bouahom et al., 2004) such as adopting agroecological practices that would make them productive and resilient in the long run.

Land shortage was reported as a major driver of food insecurity and influenced crop diversity, with the overall average household land size being 0.3ha while in the Degraded landscape the average land holding was 0.15 ha. This opens up a concern regarding the critical point at which land becomes too small to accommodate crop diversification and sustain food production let alone remain ecologically resilient (Henriksson et al., 2018; Mungai et al., 2016). This provides a huge opportunity for the implementation of agroecological principles on-farm to increase productivity while protecting the environment of such landscapes (Wezel et al., 2014).

Further, with increasing population pressure, this brings out another pertinent question regarding what complementary options are left for smallholders whose land is too small to produce enough food apart from relying on off-farm strategies. Therefore, this in return is a call to food policy makers to have a local understanding of sustainable and appropriate mechanisms to adapt to land limitations (Holden and Yohannes, 2002). This includes wholistic adoption of agroecological principles including looking beyond the farm and into the neighboring landscapes and using ecological boundaries and not administrative boundaries (Pagella and Sinclair, 2014). This will ensure that other agroecological principles such as connectivity will promote equitable and efficient distribution networks for food, while also reintegrating food systems into local economies; and putting in place mechanisms for fair trade for smallholder producers so that they benefit more significantly when purchasing food or selling their crop produce.

4.3 Promoting agroforestry adoption to enhance resilient and food secure systems

Results throughout have demonstrated the critical role that perennial crops such as agroforestry trees play a role in enhancing agroecological principles towards meeting food security needs within farming systems. Not only does having trees on farm become beneficial as trees provide numerous benefits through products such as fruits, vegetables, edible pulp, nuts; timber, fuel,

fodder, and income (Jamnadass et al., 2011). Agroforestry also plays indirect roles that help to promote ecological processes that support food production. These include: soil erosion control, soil nutrient cycling, pollination regulation, microclimate regulation, carbon sequestration and ground water recharge (Mbow et al., 2014; Minang et al., 2014; Muthuri et al., 2009). Integration of trees within farming systems therefore contributes to food security, poverty eradication and promotes livelihood and ecological resilience including climate change mitigation and adaptation (Wakaba et al., 2025). Ecological and livelihood benefits of trees are increased when there is not only higher tree diversity but also density on farms (Iiyama et al., 2017; Magaju et al., 2020).

However, in order to realize and optimize the role of agroforestry in enhancing food security, more needs to be done to address the current challenges being faced in adoption and scaling of agroforestry technologies. Studies have shown that effective scaling of agroforestry technologies in sub-Saharan Africa has been limited by various factors such as: lack of farmer participation and involvement throughout project phases from the design stage, lack of quality germplasm, and lack of tree management skills (Franzel et al., 2002; Kabwe et al., 2009). Other factors include: the inability of farmers to see tangible benefits of interventions which leads to low adoption and lack of access to markets (Bayala et al., 2010; Kiptot et al., 2007). Through initiatives from various organizations including the World Agroforestry Center (ICRAF) through the Trees for Food Security Project, these challenges are being addressed. For example, there is a move from the conventional promotion of only a few tree species were being promoted through a top-down seed and seedling systems in Rwanda. Through participatory research approaches, farmers are now being involved in selection of diverse and inclusive tree species that suit their landscapes and needs (Dumont et al., 2017). Farmers are also provided with quality germplasm and equipped with propagation skills that promotes scaling of agroforestry across the landscapes. This is supported by Figures 3 and 4, which showed an increasing number of farmers planting tree crops in 2015 compared to 1995, attributed to access to quality germplasm (66%) and the training and skills they have received from the project on tree propagation, including grafting of fruit trees (34%).

Further, results showed that soil loss through erosion was mainly reported in the Degraded landscape where unlike other landscapes, farmers reported working individually (Kuriah et al., 2019). Scaling of agroforestry requires a move from working individually at the farm/field level to working collectively at the landscape scale and beyond and working with multiple stakeholders (Sinclair, 2017). This is especially for ecological benefits such as soil erosion control and ground water recharge (Thornton et al., 2018). When the above constraints are addressed, coupled with the favorable conditions such as sloped terrain, high rainfall and collective action, there is great potential to scale agroforestry to enhance food security, thereby generating context-relevant multiple ecosystem services in Gishwati and Western Rwanda region in general.

5 Conclusions

This study revealed a significant decline in annual crop diversity in Gishwati, Rwanda, between 1995 and 2015, with some crops disappearing entirely. Farmers identified three primary drivers: the government's Crop Intensification Program (76%), which prioritized high-value crops like Irish potatoes, maize, and beans; land shortages (55%); and the abandonment of slow-growing crops (49%). These factors led to the specialization in a few high-value crops, resulting in reduced crop diversity. Consequently, 83% of farmers reported food insecurity, with seasonal food shortages (July to November) as the most common indicator (51%), followed by fewer meals (47%), consuming less-preferred foods (22%), and reducing portion sizes (14%). Perennial crops, particularly fruit trees, played a critical role in bridging hunger gaps during food-insecure periods.

The study highlights the importance of increasing crop diversity by integrating annual and perennial crops, including those considered “low-value,” to enhance food and nutritional security. Significant variations were observed in crop diversity, food availability trends, and food insecurity indicators across degraded, recovering, and restored landscapes, underscoring the need for context-specific interventions tailored to land degradation status. The research identified seven agroecological principles—biodiversity, synergy, economic diversification, social values and diets, soil health, and participation—that are critical for addressing the crop diversity–food security–land degradation nexus. Food produced on-farm sustained households for only 6.6 months in 2015, down from 10.1 months in 1995, increasing reliance on off-farm food sources. This reliance indicates systemic gaps, where short-term solutions hinder long-term investments in farming systems and sustainable food production. To address these challenges, holistic promotion of agroecological principles is essential. This includes leveraging ecological rather than administrative boundaries, ensuring connectivity within food systems, and fostering equitable trade mechanisms for smallholder farmers. The study also highlights opportunities to implement agroecological practices on small farms (average size 0.3 ha) to enhance productivity and environmental protection. However, it raises concerns about the minimum land size needed to sustain crop diversification and ecological resilience.

In conclusion, the study calls for food security policies to embrace both crop diversity alongside specialization and ensure the interventions are adapted to local contexts. Findings from this study have been validated and supported through numerous literature and studies over time. Therefore, incorporating co-creation of knowledge by integrating local and scientific knowledge into agroecological food policies can ensure context-appropriate, inclusive, and sustainable solutions, fostering resilience in smallholder farming systems and advancing transitions to sustainable food systems.

6 Limitations of the study

While this study offers critical insights into the agroecological transitions in Rwanda and the role of local knowledge in

understanding the crop diversity–food security–land degradation nexus, it has several limitations:

1. **Scope and Temporal Scale:** The study relies on data spanning from 1995 to 2015. While this provides a long-term perspective, it does not capture recent developments, including recent policy changes and their impact on crop diversity and food security.
2. **Geographical Coverage:** This research focuses on Gishwati, Rwanda, as a case study, which may limit the generalizability of findings to other regions with different agroecological and policy contexts.
3. **While local knowledge is a prerequisite for designing contextualized solutions for crop diversification–food security nexus, additional methodologies such as policy engagement to bridge the gap between local knowledge recognition and actionable policy recommendations.**

To build on these findings, future research could focus on the following areas:

1. **Expanding Longitudinal Studies:** Extending the timeframe of analysis to include more recent data will help capture current agroecological trends and evaluate the long-term effectiveness of policy shifts.
2. **Comparative Studies Across Agroecological Zones:** Conducting comparative studies in different agroecological zones and policy environments would enhance understanding of how contextual factors influence agroecological transitions.
3. **Future research could also focus on developing and testing participatory policy engagement frameworks that effectively integrate local knowledge into actionable policy recommendations. This could involve exploring co-creation processes between farmers, policymakers, and researchers to bridge the gap between local knowledge recognition and the formulation of policies that support crop diversification and food security.**

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: <https://dataverse.harvard.edu/dataset.xhtml?persistentId=doi:10.7910/DVN/YQJ0SH>.

Ethics statement

The studies involving humans were approved by Bangor University Research Ethics Committee. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written

informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

AK: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. TP: Conceptualization, Investigation, Methodology, Supervision, Validation, Visualization, Writing – review & editing. CM: Conceptualization, Methodology, Project administration, Supervision, Validation, Writing – review & editing. FS: Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing.

Funding

The author(s) declare that financial support was received for the research and/or publication of this article. This study is partially funded by the Transformative Partnership Platform on Agroecology (Agroecology TPP) and partially by the Australian Centre for International Agricultural Research (ACIAR) under the Improving sustainable productivity in farming systems and enhanced livelihoods through adoption of evergreen agriculture in eastern Africa shortened as “Trees for food security project, Grant Number FSC/2012/014 that was led and managed by the World Agroforestry (ICRAF) under the CGIAR Research Program on Forest, Trees and Agroforestry (FTA).

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Acknowledgments

The authors would like to express their gratitude to the farmers in Gishwati, Western Rwanda who generously dedicated their time to share their knowledge. They also extend their appreciation to the translators who assisted during data collection, the local administrative authorities in the study areas, and partners for their valuable support. Also acknowledged is the ICRAF Rwanda country office, led by Dr. Athanase Mukuralinda—country representative for their hosting and logistical support during data collection period.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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OPEN ACCESS

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RECEIVED 26 November 2024

ACCEPTED 12 March 2025

PUBLISHED 10 April 2025

CITATION

Rusch HL, Hunter MC, Kraus A, Tautges NE
and Jungers JM (2025) Intermediate
wheatgrass as a dual use crop
for grain and grazing.
Front. Agron. 7:1534962.
doi: 10.3389/fagro.2025.1534962

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Intermediate wheatgrass as a dual use crop for grain and grazing

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Introduction: Intermediate wheatgrass [*Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey] (IWG) is a novel perennial grain crop with the potential for dual use (DU) in a system that includes the harvest of summer grain and straw as well as the grazing of crop regrowth. This could diversify grower income streams but impacts on productivity and profitability of DU systems need evaluation.

Methods: A 4-year on-farm trial was conducted in Minnesota, USA comparing yields and net revenue of a grain+straw production system (GP) vs. a DU system. For both the GP and DU systems, the grain and straw yields from the summer harvest were evaluated, the subsequent IWG regrowth was measured in the fall and again in spring to quantify forage production and nutritive value, and the economic value of grain, straw, and forage were calculated. In the DU system, the herbage intake and forage utilization were also studied.

Results and discussion: The GP system produced 42% more grain and 41% more straw than the DU system in year 2 but both systems produced similar grain and straw yields in year 3. The DU system produced greater grain yields than the GP in year 4. Across systems, the forage yield peaked in year 3. Both agronomic systems generally displayed similar forage yields of comparable nutritive value. Crude protein (CP) in fall and spring forage averaged 140 to 150 g kg⁻¹ whereas CP was 30 g kg⁻¹ in the summer straw, comparable to common annual small grains. The relative feed value of IWG forage in the fall was 100 and 127 in spring compared with 80 in the summer. The sale of higher year 2 grain yields in the GP system led to this system earning a net return to the enterprise of \$721 ha⁻¹ yr⁻¹ with the DU system producing \$609 ha⁻¹ yr⁻¹. In conclusion, grazing IWG can take advantage of on-farm forage resources to generate revenue but waiting to begin grazing until after the second-year grain harvest may reduce the risk of grain and straw yield losses to enhance net returns to the enterprise.

KEYWORDS

Thinopyrum intermedium, perennial grain, nutritive value, enterprise budget, forage yield

1 Introduction

Intermediate wheatgrass [*Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey] (IWG) is a cool-season perennial grass in development as a perennial grain crop. Initially introduced into the U.S. as a forage crop, IWG provides multiple ecosystem services, including continuous living ground cover that prevents soil erosion (Kantar et al., 2016) as well as extensive rooting systems with the potential to accrue soil C (van der Pol et al., 2022) and reduce soil nitrate leaching (Jungers et al., 2019; Reily et al., 2022). Yet, adoption of a new perennial grain crop that provides environmental benefits and meets demand for sustainable food products also introduces economic risks to the farmer. Compared with conventional annual grains like wheat (*Triticum aestivum* L.), IWG produces lower grain yields. Studies in Michigan and New York reported that IWG produced 4.5%, 17%, and 33% as much grain as annual wheat (Law et al., 2022; Culman et al., 2023). Furthermore, a lack of crop insurance, consistent market demand, and accessible supply chain infrastructure for new crops disincentivizes production and stifles industry. However, identification of a secondary revenue stream from a new crop, such as forage in the case of IWG, can mitigate these economic barriers and facilitate the expansion of new crops.

Unlike annual crops that require planting each year, the perenniality of IWG enables vegetative growth early in the spring and late into the fall when annual fields lie fallow. Managing this vegetative growth for forage or hay production can generate additional revenue in a grain-type IWG operation (Hunter et al., 2020b). The relative value of forage in the system may increase over time since IWG grain yields (and thus sales) decline as the stand matures, so forages may contribute a greater portion of total revenues (Zhen et al., 2024). Additionally, the crop residue at grain harvest can be sold as straw for animal bedding or mixed into rations for beef or dairy cow feed. Thus, IWG vegetation could be commercialized as forage for feed or fodder up to three times per year, once in the spring, once in summer, and once in the fall. A Wisconsin study reported that forage economic value of IWG accounted for up to 40% of the potential total revenue in a dual use (DU) system that produced both grain+straw and forage (Pinto et al., 2022). The latter highlights the potential productive and economic contribution of forage in DU systems, which may improve on-farm resource use efficiency and profitability compared with grain+straw production (GP) systems, at least until grain yields are improved through breeding efforts.

Several recent studies investigated the production and profitability of grain-type IWG for DU production of grain+straw and hay (Culman et al., 2023; Law et al., 2022, Law et al., 2021; Hunter et al., 2020a, Hunter et al., 2020b). Across nine North American locations, an IWG DU system with a fall hay harvest led to greater grain yields than in the GP system with only a summer grain+straw harvest, although a spring (instead of a fall) hay harvest reduced the grain yield (Culman et al., 2023). In Minnesota, researchers studying three grain-type IWG DU systems with different hay harvest frequencies (spring only, fall only, and spring+fall) did not observe grain or straw yield declines in fall only and spring+fall DU systems compared with the control system

(i.e., no hay harvest) while the spring only DU system sometimes reduced summer grain and straw yields because stands did not fully recover following May defoliation to produce grain to their highest potential (Hunter et al., 2020a; Hunter et al., 2020b). Fall only and spring+fall DU systems reported similar total forage (straw + hay), which were greater than the total forage yield in spring only DU system and in the control system (Hunter et al., 2020b). These studies underscore the variable effect of hay harvests on grain and forage yields in IWG DU systems.

In terms of nutritive value, IWG produces forage of comparable quality to barley (*Hordeum vulgare* L.), oat (*Avena sativa* L.), or wheat. The crude protein (CP) of IWG cut for hay ranged from 105 to 132 g kg⁻¹ in fall and 195 to 288 g kg⁻¹ in the spring (Hunter et al., 2020b) compared with the CP of mature oat and spring barley harvested approximately 100 days after planting, which were 109 g kg⁻¹ and 105 g kg⁻¹ in a study conducted in Turkey (Kocer and Albayrak, 2012). Similar values were reported for oat and barley cut for forage at the hard dough stage, which had CP values of 112 and 103 g kg⁻¹, respectively (Pursley et al., 2020). The relative feed value (RFV) of IWG cut for hay ranged from 89 to 107 in the fall and 147 to 161 in the spring (Hunter et al., 2020b) compared with RFV of 97 for oat, 85 for barley, and 88 for wheat at the milk dough stage (Yavuz and Gulumser, 2022). The RFV of IWG straw harvested in the summer along with grain ranged from 57 to 70 (Hunter et al., 2020b) whereas the RFV of wheat straw was reported at 47 elsewhere (Kaithwas et al., 2020). Using RFV as a predictor in a model trained on recent hay sales, Hunter et al. (2020b) predicted the sale prices of the three types of forage (straw and hay). These prices reflected the same seasonal ranking, however, the summer straw produced three to four times as much biomass as the spring and fall hay harvests (Hunter et al., 2020b). So, straw was a more valuable product than the more nutritious but less abundant hay. Nonetheless, IWG regrowth in the spring and fall provides a source of highly nutritious forage at a time of limited forage resources and an additional income stream.

The literature on DU systems for grain and hay highlights potential tradeoffs between these and GP systems. Considering that important differences exist in the defoliation method of forage managed for hay (homogeneous) vs. grazing (heterogeneous) there may be limitations to the application of the results from one type of management to the other. Thus, the present study compared the productive and economic potential of a grain-type IWG system for GP vs. a DU system involving a summer grain +straw harvest and grazing of IWG regrowth in the fall and spring. The objectives were to evaluate the grain, straw, and forage yields; harvest index; nutritive value; and enterprise budgets of these two agronomic systems, as well as the herbage intake (HI) and forage utilization (FU) of the DU system. It was hypothesized that 1) the DU system would produce as much or more grain and straw than the GP system, although yields were expected to decline with increasing stand age in both systems, 2) the DU system would produce greater forage yields than the GP system, 3) fall would produce greater forage yields and have greater HI and FU than spring, 4) forage CP and RFV would be greater in spring and fall compared with summer straw, thus DU system would have superior

nutritive value overall than the GP system, and 5) the DU system would be as profitable or more profitable than the GP system given the additional revenue stream from grazing.

2 Materials and methods

2.1 Study site and experimental design

A four-year on-farm trial was conducted from September 2018 to August 2022 near Goodhue, Minnesota, USA (44°24'02"N, 92°37'26"W; 335 m.a.s.l.) to compare the production and profitability of an IWG GP system (ungrazed control) with a DU system of IWG for both grain+straw and grazing. The soil was mapped as a Knox silt loam (fine-silty, mixed, superactive, mesic Mollic Hapludalfs). Soil tests in September 2018 indicated that N, P and K levels were more than adequate at the 0-15 cm depth and averaged 27, 52 and 419 ppm, respectively. Soil pH was 7.4 and OM 3.7%. Monthly minimum and maximum air temperature and total precipitation data were collected from the nearest National Weather Service Reporting Station in Zumbrota, Minnesota, US (44°17'59"N, -92°39'56"W; 344 m.a.s.l.; Minnesota Department of Natural Resources, 2025). Missing weather data points were supplemented with time series data from the Oregon State University PRISM Climate Group for Goodhue county, Minnesota, US (44°24'36"N, -92°43'21"W; 359 m.a.s.l.; PRISM Climate Group, 2025).

The study was established as a completely randomized design consisting of three replications with the two agronomic systems (GP and DU) represented in each. Each replication was a 0.8 ha paddock with an enclosure area approximately 60 m². Electric fencing was used to create the enclosure area for the ungrazed control treatment (GP system). The remainder of each paddock was assigned to the DU treatment to allow grazing cattle access to forage in the fall and spring each year.

2.2 Management

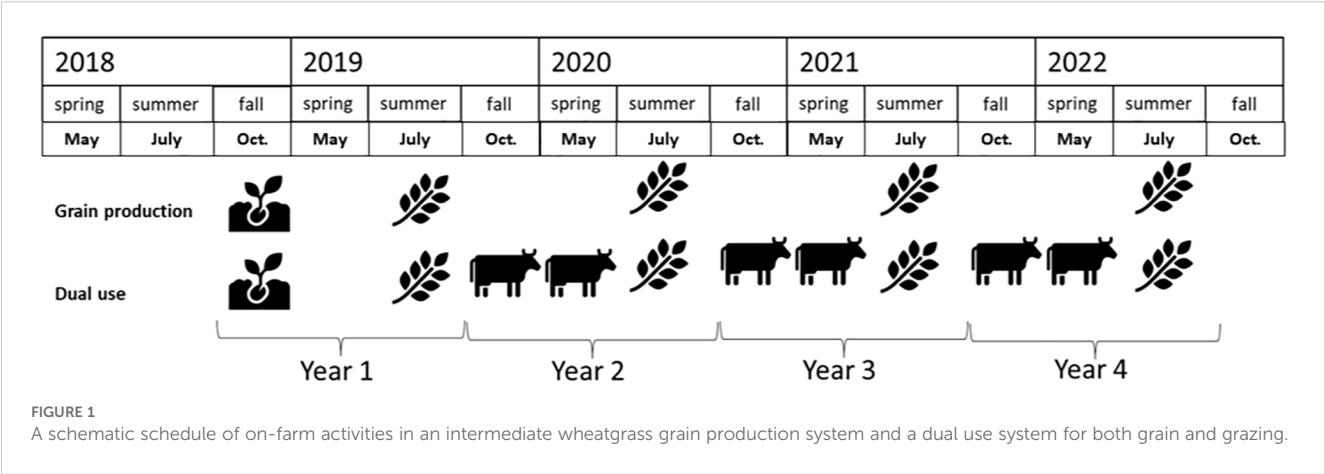
The entire experimental area was previously a mixed species grass pasture, which was terminated on September 2, 2018 with

glyphosate applied at labeled rates. Seed from an advanced breeding population of grain-type IWG procured from the University of Minnesota breeding program was planted in rows 19cm apart at a seeding rate of 20 kg pure live seed ha⁻¹ using a no-till drill on September 10, 2018. By spring 2019, the IWG was well established. Herbicide was applied on July 3, 2019 using 2,4-D at labeled rates for a perennial grass. For fertility management, 18,927 L ha⁻¹ of liquid dairy manure was applied each summer. This fertilization, which was repeated in the spring and summer of years 3 and 4, supplied approximately 45 kg N ha⁻¹ yr⁻¹, 23 kg P ha⁻¹ yr⁻¹, and 45 kg K ha⁻¹ yr⁻¹. The DU treatment may also have benefited from cattle urine and manure deposition during the grazing period.

2.3 Data collection

The first data collection occurred with grain and straw harvest on August 23, 2019, approximately one year after establishing IWG. Hereafter year refers to the IWG stand age in years after establishment beginning in the fall (September) and ending in the summer of the subsequent calendar year (August; Figure 1). One subsample of grain and straw per experimental unit was collected by hand using a 0.5 m² quadrat prior to production-scale grain harvesting in July/August each year. In the hand harvest, seed heads were separated from the stems and leaves in the subsample, the grain, stems and leaves were then weighed, dried at 60°C, and straw dry matter yields recorded. Grain was threshed from seed heads using a laboratory thresher (Wintersteiger LD-50), aspirated to remove chaff, and weighed to determine grain dry matter yield. Grain samples were about 80% dehulled. Summer experimental grain and straw yields reported are from the hand harvested subsamples. Grain and straw yields from hand-harvested quadrats are referred to hereafter as “experimental” to differentiate these yield estimates from those derived during the production-scale harvest by the producer. The harvest index was calculated as the dry grain weight divided by the sum of the dry grain weight plus the dry straw weight.

After hand sampling manually, the electric fence enclosures were removed to harvest the remaining grain and straw from the entire paddock. The grain was swathed and then picked up by a



combine with a pickup header, except in 2019, when it was directly combined (John Deere, Moline, Illinois, USA). Straw was collected along with grain except in 2022. Electric fence enclosures were subsequently replaced using GPS coordinates. The grain and straw harvested mechanically was reported in the farm enterprise budget and is referred to hereafter as the “farmer” grain and straw yield.

The grazing treatment was first implemented in the DU system as the IWG stand entered its second year after establishment in mid-October 2019. Written informed consent was obtained from the owners for the participation of their animals in this study. The farm owner managed the grazing herd such that each paddock was grazed by 31 cow-calf pairs (~1.7 AU) plus 2 heifers (~1.3 AU each), at a stocking density of 560 kg LW ha⁻¹, for five to twelve days in October/November 2019, 2020, and 2021 and in May 2020, 2021, and 2022 until a targeted range of 60% forage removal was achieved. Forage biomass was estimated in both the GP and DU systems by hand clipping the vegetation to an 8cm stubble height from three 30cm × 30cm quadrats per paddock to simulate mowing. Experimental samples were collected from both systems on the same day each year based on when grazing would occur in the DU system such that forage was evaluated on a date before grazing (pre-graze) and after grazing (post-graze). Fresh experimental forage weights were recorded before placing samples in a forced air oven to be dried at 60°C until moisture was removed from the biomass at which point the dry weights were recorded and are reported as experimental forage yield. Herbage intake (HI) was calculated as the difference between the pre-graze and post-graze experimental forage biomass (Smart et al., 2010). While the high grazing pressure asserted on the paddocks was assumed to have produced a decline in herbage post-grazing, the potential for uneven grazing as well as for active growth of IWG between these two sampling events may diminish the extent to which herbage declined. Forage utilization was calculated by dividing HI by the pre-graze experimental forage biomass to get the percentage of the total forage grazed (Smart et al., 2010).

A subsample of each of the dried experimental straw (summer harvest) and experimental forage (fall and spring harvests) biomass were ground through a 6mm screen in a Wiley mill (Thomas Scientific, Swedesboro, NJ) and subsequently through a 1mm screen in a Cyclotec (Foss, Hillerød, Denmark) before scanning under near infrared reflectance spectroscopy using a FOSS NIRS (Perkin Elmer DA7250, Waltham, Massachusetts, USA) with calibration equations developed with Minnesota IWG to estimate CP, acid detergent fiber (ADF), and neutral detergent fiber (NDF). The RFV of the experimental straw and experimental forage was calculated using Equations 1–3 (Moore and Undersander, 2002):

$$\text{Dry matter intake (DMI)} = 120/\text{NDF} \quad (1)$$

$$\text{Digestible dry matter (DDM)} = 88.9 - (0.779 \times \text{ADF}) \quad (2)$$

$$\text{RFV} = (\text{DMI})(\text{DDM})/1.29 \quad (3)$$

where NDF and ADF are a percent of dry matter.

2.4 Farm enterprise budget

Based on actual on-farm expenses and revenues from the management and sale of farmer grain, straw, and grazing forage, three enterprise budget scenarios for the production and sale of IWG were examined: grain only (GR), grain+straw (GP), and grain+straw+forage (DU). Although from a management perspective removing the straw along with the grain is important, the GR scenario illustrates sales if straw were not marketed. Some discrepancies arose between the yields reported by the farmer and the experimental yields obtained by hand-harvesting with quadrats. In general, the farmer's yields were somewhat lower than the experimental yields, likely due to some seed loss from seed shatter during the mechanical harvest. As an established metric for valuing forage, the RFV was used as a proxy to estimate the market price that the farmer's IWG straw and forage might earn, however, as a novel crop, the markets for IWG are still in development.

The net return to the enterprise was calculated as the total revenues less the total expenses. Net returns were standardized and reported on a per hectare per year basis. This standardization annualizes one-time expenses like seed and planting costs, which only occurred during the establishment year. Similarly, the standardization summarizes the farmer's grain, straw, and forage yields and revenues, which were averaged across the four years of the production, including the year of establishment (year 1 = 2018) when no production or sales occurred. The zero for year 1 farmer grain, straw, and forage yields draws down the average farmer yields in the farm enterprise budget. Thus, some discrepancies may be perceived between the standardized farmer's yields reported in the farm enterprise budget compared with the annual experimental yields presented elsewhere in the manuscript, which are reported on a per hectare basis for each of three years of treatment implementation and excludes the establishment year.

2.5 Statistical analysis

All statistical analyses were performed using R version 4.3.2 (R Core Team, 2023). Linear mixed effects models were run for all analyses using the *nlme* package (Pinheiro et al., 2023). Across all models, replication and plot were treated as random effects. Fixed effects for the experimental grain and straw yields, and harvest index models included stand age and agronomic system (i.e., treatment). The agronomic system was not included in the model for HI and FU as only the DU system was grazed, so data for analysis was only analyzed for this system. For the experimental forage yield, HI, FU, CP, and RFV models the fixed effects included season. All models were run with and without a variance structure and correlation structure and the Akaike's Information Criterion (AIC) was used to select the model of best fit. The grain and harvest index models specified the 'varIdent()' variance structure to account for the heterogeneity introduced by stand age. Similarly, the variance structure specification was applied for season in the models for HI, FU, CP, and RFV. A correlation structure

(corAR1) was specified in the experimental forage yield and RFV models to account for repeated measures in the fall and spring each year. All models were optimized using the nlmeControl() specification. This manuscript reports the analysis of variance and pairwise comparisons of estimated marginal means from *emmeans* (Lenth, 2023) for each model. Statistical results are presented using *p*-values to discuss differences according to hypothesis tests and include 95% confidence intervals in figures to illustrate the variation in the data around mean estimates.

The following missing data were excluded from statistical analyses. Data for 2018 (year 1) experimental grain and straw yields were excluded because these harvests occurred prior to implementing the DU system (i.e., grazing treatment). Experimental forage yield, HI, and FU data for fall and spring for year 1 were excluded from the statistical analysis for the same reason. In 2022 (year 4), the experimental straw data was excluded from the analysis of experimental straw yield due to missing experimental straw yield data for both agronomic systems that year. For the same reason, the analysis of CP and RFV were excluded in year 4.

The models for the net return to the farm enterprise budget included the three enterprise budget scenarios (GR, GP, DU) as the fixed (treatment) effect and the year was treated as a random effect.

3 Results

3.1 Weather

For the study period, the average annual minimum and maximum air temperatures were 2°C and 12°C, respectively, which were similar to the 30-year normals (Table 1). The average monthly cumulative precipitation during the study period was 920 mm while the 30-year normal was 873 mm. These averages mask the wide range of variation in temperature and precipitation during any given year at the study site. For example, in year 3 the minimum air temperature reached as low as -20°C while highest monthly average air temperature that year was 29°C. Thus, within the span of one year, the range of air temperatures spanned a range of 49°C. The range of air temperatures spanned 42°C in year 1, 43°C in year 2, and 48°C in year 4. Over the past 30 years air temperature spanned a range of 40°C between the lowest minimum and highest maximum monthly average air temperatures. So, a wider range of air temperatures was observed in the present study than for the 30-year average.

In general, the average cumulative precipitation by month in the first two years of the study was greater than the 30-year average for the study site while the last two years of the study were drier than normal (Table 1). In the establishment year, the monthly

TABLE 1 Minimum and maximum average monthly air temperatures and cumulative monthly precipitation for the study years.

	Production season												Annual average
	Fall			Winter			Spring			Summer			
	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	
Average minimum temperature (°C)													
30yr average	10	3	-4	-11	-14	-13	-6	1	8	14	16	14	2
Year 1 (establishment)	12	1	-8	-9	-16	-19	-10	1	6	13	17	13	1
Year 2	13	2	-6	-11	-11	-14	-2	-1	7	14	16	14	2
Year 3	9	-1	-4	-10	-12	-20	-4	0	7	15	13	13	1
Year 4	8	5	-4	-10	-20	-17	-7	-2	8	14	16	14	0
Average maximum temperature (°C)													
30yr average	23	16	6	-1	-4	-2	6	14	20	26	28	27	12
Year 1 (establishment)	23	12	0	-1	-6	-7	2	13	17	25	28	25	11
Year 2	24	13	2	-1	-3	-2	6	12	19	28	29	27	13
Year 3	21	10	7	1	-2	-8	9	13	20	29	28	27	13
Year 4	24	18	7	0	-6	-4	6	10	21	27	28	26	13
Average precipitation (mm)													Total annual
30yr average	91	63	40	33	26	27	48	80	109	138	98	120	873
Year 1 (establishment)	200	115	43	44	26	87	52	110	150	144	194	65	1,230
Year 2	183	136	54	33	20	18	40	36	171	157	64	160	1,072
Year 3	61	47	51	19	25	12	42	21	109	38	44	221	690
Year 4	42	49	34	23	14	13	47	67	101	60	70	104	688

cumulative precipitation was greater than the 30-year average every month, except for August, which was 55 mm below average for that month. In the planting month (September) of the establishment year, the precipitation was 109 mm above and the minimum air temperature was 13°C greater than the 30-year average for the same month. Conversely, in year 3 the cumulative monthly precipitation was below average every month except for November (+10 mm) and August (+101 mm), with the lowest precipitation recorded in June and July at 100 mm and 54 mm below the 30-year average, respectively. The range in the 30yr-average precipitation was from 26 mm in January to 138 mm in June, a range of 112 mm. In the present study, the difference between the month with the most and the least precipitation ranged from 209 mm between August (221 mm) and February (12 mm) in year 3 to a difference of 91 mm in year 4 between August (104 mm) and February (13 mm). Overall, the weather observed in the present study varied more than historical averages.

3.2 Summer experimental grain and straw yields and harvest index

The interaction of stand age $\times$ agronomic system ($p < 0.001$) explained differences in observed experimental grain yields. Experimental grain yield in the establishment year (year 1) was not considered in the analysis because the grazing treatment was not implemented until year 2, thus the two agronomic systems in year 1 could not be compared. As a point of reference, though, the GP system produced an experimental grain yield of 990 kg ha⁻¹ in

year 1. In year 2, a greater experimental grain yield ($p < 0.01$) was observed in the GP system (1,010 kg ha⁻¹) than in the DU system (710 kg ha⁻¹; **Figure 2a**). In year 3, the experimental grain yield was similar among agronomic systems ($p = 0.44$). In year 4, the experimental grain yield was greater in the DU system than in the GP system ($p = 0.04$). Specifically, the DU system yielded 533 kg ha⁻¹, or 27% more grain than the GP system. The DU system produced as much and more IWG grain than the GP system in two out of three years, which supported hypothesis #1. However, overall, the DU system produced 14% less total experimental grain (1,822 kg ha⁻¹) than the GP system (2,081 kg ha⁻¹). The results also indicate a decline in experimental grain yields over time, particularly in the GP system. From year 2 to year 4, experimental grain yield declines in the DU system were 25% and 62% in the GP system.

There was an interaction between stand age $\times$ agronomic system ($p < 0.0001$) that impacted IWG experimental straw yields and was driven by the lack of difference among year 2 and year 3 experimental straw yields in the DU system, compared with the greater variability in experimental straw yields among year 2 and year 3 stands in the GP system. The DU system produced 3.8 Mg ha⁻¹ less experimental straw than the GP system in year 2 ($p < 0.0001$) but similar experimental straw yields of approximately 5 Mg ha⁻¹ were observed in both agronomic systems in year 3 ($p = 0.49$). Overall, the DU system produced 29% less experimental straw than the GP system across the two study years, due to lower year 2 experimental straw yield. Intermediate wheatgrass experimental straw yields declined with stand age in the GP system (**Figure 2b**). In the GP system, experimental straw yields

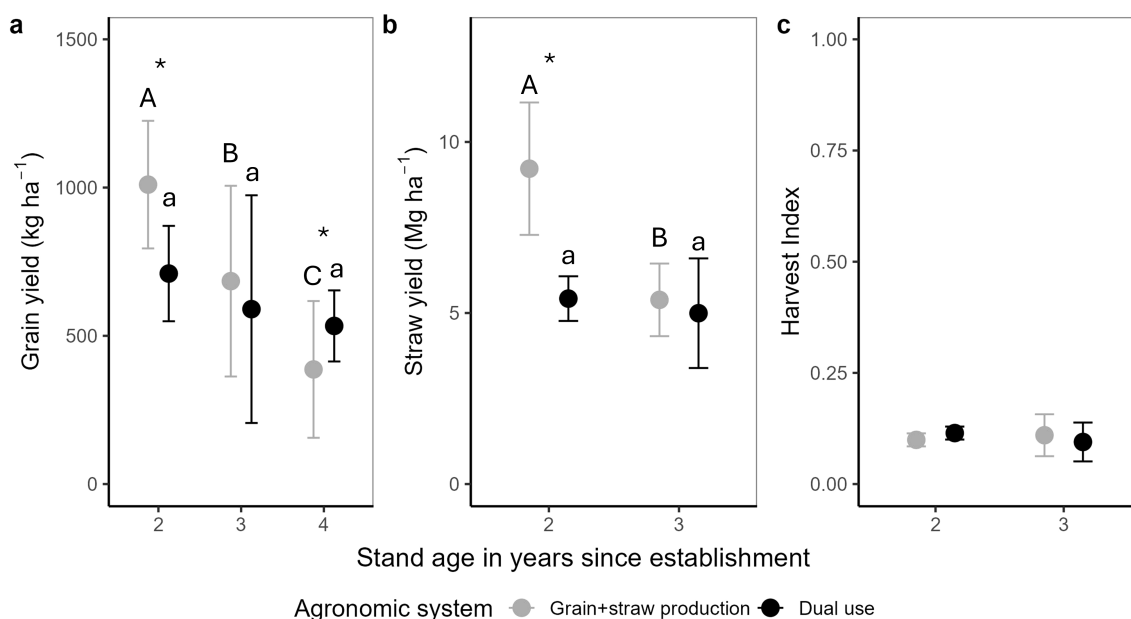


FIGURE 2

Mean experimental yields of intermediate wheatgrass (IWG) (a) grain and (b) straw harvested from a system for grain+straw production (GP) and a dual use (DU) system for grain+straw and grazing forage as well as the (c) harvest index for both agronomic systems. Straw yield was not measured in year 4. Within a given agronomic system, significant differences ($p < 0.05$) between IWG stand ages are indicated by different upper-case (GP) and lower-case (DU) letters. Within a given IWG stand age, an asterisk indicates a significant difference between agronomic systems. Points represent calculated means and vertical lines represent 95% confidence intervals.

declined by 42% from 9.2 Mg ha⁻¹ in year 2 to 5.4 Mg ha⁻¹ in year 3. Meanwhile, the experimental straw yield in the DU system averaged 5.2 Mg ha⁻¹ across the two study years. Taken together, the DU produced as much or more experimental grain and straw as the GP system, as hypothesized, but only after year 2.

No differences in harvest index were observed between the GP and DU systems in years 2 or 3 ($p = 0.10$). The harvest index averaged across agronomic systems and stand ages was 0.15 (Figure 2c).

3.3 Experimental forage yield, herbage intake, and forage utilization

Differences in experimental forage yield arose from the interactions of season $\times$ agronomic system ($p < 0.0001$) and stand age $\times$ season ($p < 0.0001$). However, it is important to note that while experimental forage production in the GP system was quantified by hand cutting, it was not grazed, thus GP forage yields represent potential experimental forage yield (i.e., the amount of forage available that was not consumed) rather than the realized experimental yield as in the DU system. The experimental forage yield for a given agronomic system varied by season (Figure 3a). Averaged across years, the realized experimental forage yield in the DU system in fall was 0.58 Mg ha⁻¹ greater than in the spring ($p < 0.0001$). Contrary to the hypothesis, no differences between agronomic systems were observed for the experimental forage yield in fall ($p = 0.39$) or spring ($p = 0.07$).

Among production years, year 3 experimental forage yields were unlike those produced in years 2 and 4 (Figure 3b). The experimental forage yield in the fall of year 3 was 1.14 Mg ha⁻¹ greater than in the fall of year 2 ($p < 0.0001$) and 1.16 Mg ha⁻¹ greater than in the fall of

year 4 ($p < 0.0001$). Conversely, the experimental forage yield in the spring of year 3 was 0.67 Mg ha⁻¹ less than in the spring of year 2 ($p < 0.001$) and 0.45 Mg ha⁻¹ less than in the spring of year 4 ($p = 0.03$). Meanwhile, the experimental forage yields in years 2 and 4 were similar in both fall ($p = 0.99$) and spring ($p = 0.42$).

Herbage intake (HI), the amount of forage consumed by grazing animals over a given unit area (Smart et al., 2010), was impacted by the interaction of stand age $\times$ season ($p < 0.0001$). This was driven by differences in the fall HI in year 3 being more than twice that of the fall HI in year 2 ($p < 0.0001$) and year 4 ($p < 0.001$; Figure 4a). Additionally, greater HI was observed in fall than in the spring in year 3 ($p < 0.0001$) and year 4 ($p = 0.04$). In year 3, the HI declined 95% from 2.29 Mg ha⁻¹ in the fall to 0.11 Mg ha⁻¹ in the spring (Figure 4a). Similarly, in year 4 the spring HI (0.46 Mg ha⁻¹) was 56% lower than the fall HI (1.05 Mg ha⁻¹). Thus, we observed evidence to support the hypothesis that fall HI would be greater than spring HI in two of three study years.

Forage utilization (FU), the proportion of available forage that is consumed or destroyed by grazing animals expressed as a percentage (Guretzky et al., 2020; Smart et al., 2010), reflected the trends for HI (Figure 4b). As in HI, the stand age $\times$ season interaction ($p = 0.01$) influenced FU. Specifically, the difference between the fall FU (92%) and spring FU (12%) in year 3 ($p < 0.0001$) drove this result and provided evidence in support of hypothesis #3.

3.4 Forage nutritive value

The CP in experimental forage varied by stand age $\times$ season ($p < 0.0001$). Averaged across agronomic systems, year 2 CP in the fall experimental forage was 212 g kg⁻¹ and declined by 75 g kg⁻¹ in

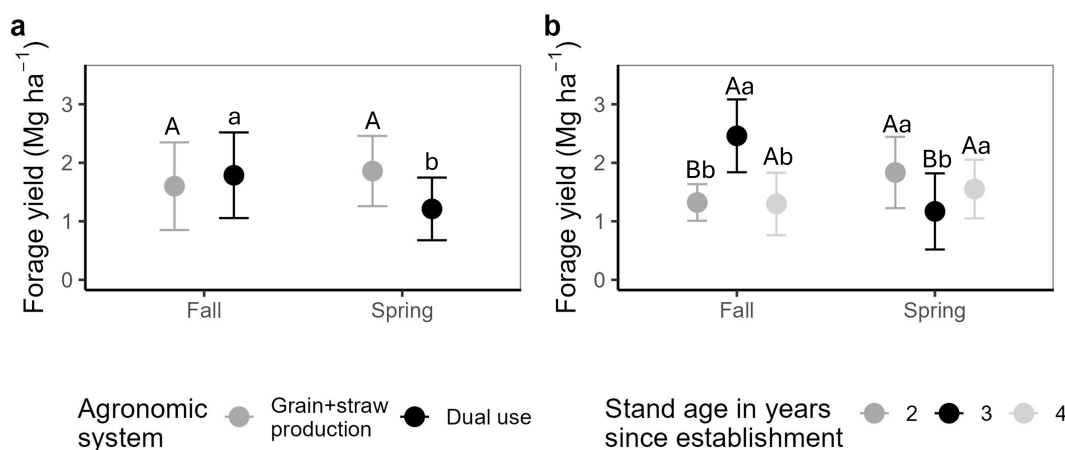


FIGURE 3

Intermediate wheatgrass (IWG) vegetative growth available as forage for grazing (a) in two agronomic systems during the fall and spring. Values for the grain+straw production (GP) system represent potential experimental forage as no grazing occurred in this system while values for the dual use (DU) system, in which grazing did occur, represent realized experimental forage yields. For a given agronomic system, significant differences ($p < 0.05$) in experimental forage yield between seasons are indicated by different upper-case (GP) and lower-case (DU) letters. (b) Seasonal experimental forage yield by IWG stand age. For a given IWG stand age, different upper-case letters indicate significant differences in experimental forage yield between seasons. Within a given season, different lower-case letters indicate a significant difference in experimental forage yield between IWG stand ages. Points represent calculated experimental means and vertical bars represent 95% confidence intervals.

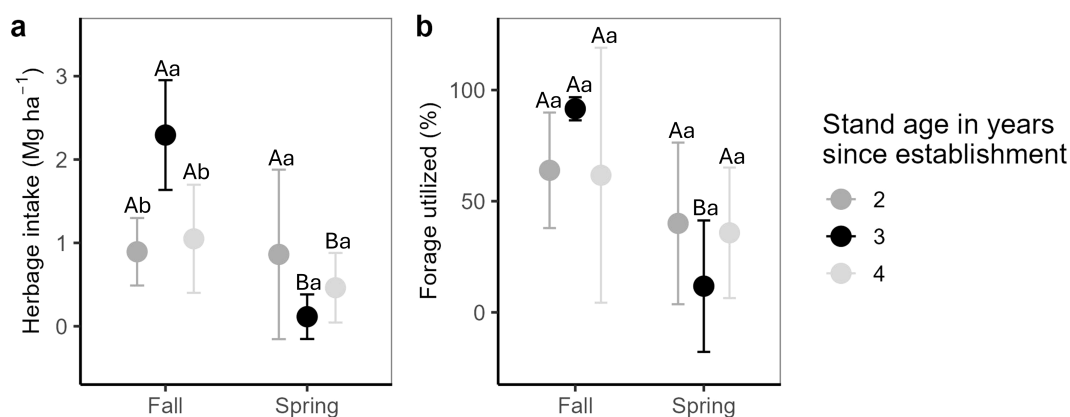


FIGURE 4

Means for the intermediate wheatgrass (IWG) (a) herbage intake and (b) forage utilization by grazing cattle as a proportion of the available forage. Within a given IWG stand age, significant differences ($p < 0.05$) between seasons are indicated by different upper-case letters. Within a season, different lower-case letters indicate a difference between IWG stand ages. Points are calculated means and vertical bars represent 95% confidence intervals.

spring experimental forage, with summer experimental straw containing 87% less CP than the fall experimental forage that year (Figure 5a). Thus, the CP ranking in year 2 was fall>spring>summer. In year 3, however, the CP ranked spring>fall>summer. The fall experimental forage CP in year 3 was under 70 g kg^{-1} in both agronomic systems, a 70% decline from the previous year. Meanwhile, the spring experimental forage remained relatively stable across years and agronomic systems.

Differences in RFV arose from the interaction of stand age $\times$ season ($p < 0.0001$), season $\times$ agronomic system ($p = 0.002$), and the

three-way interaction of stand age $\times$ season $\times$ agronomic system ($p = 0.04$). No differences in RFV were observed among agronomic systems, except for in the spring of year 3 ($p < 0.01$) when the RFV of spring forage in the DU system was 21 units greater than in the GP system. Within an agronomic system, there were seasonal variations in RFV (Figure 5b). In the GP system in year 2, the summer RFV was 50 units less than in the fall ($p < 0.0001$) and 61 units less than in the spring ($p < 0.0001$). In year 3, the RFV of the experimental forage in the GP system was greater in the spring than in the fall ($p < 0.0001$) and the summer straw RFV was the lowest

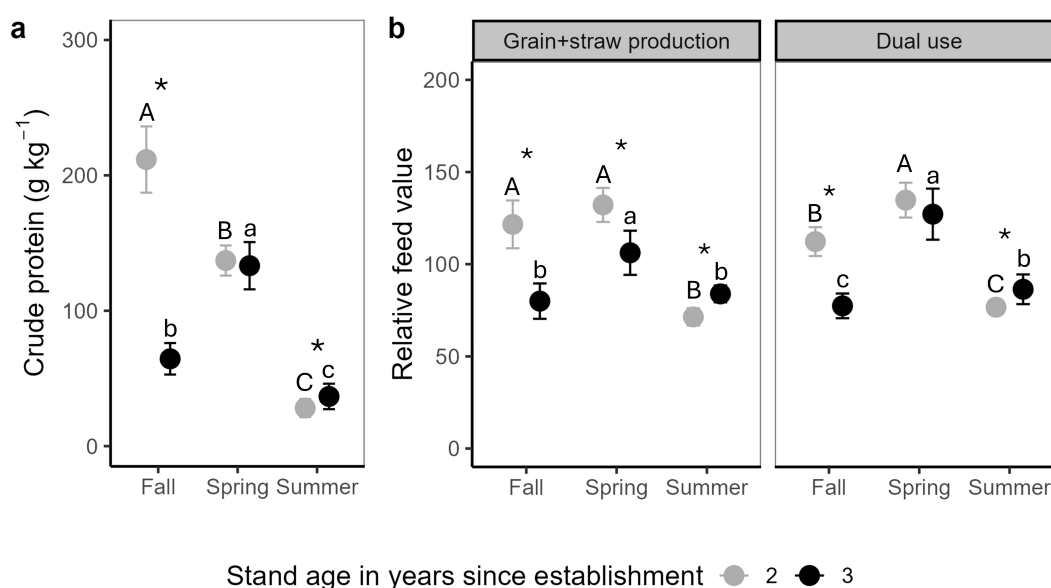


FIGURE 5

The seasonal nutritive value in terms of (a) crude protein and (b) relative feed value of intermediate wheatgrass (IWG) vegetative growth available as forage for grazing in fall and spring and as straw in summer in a grain+straw system and the dual use system for grain+straw production and grazing of forage. Different letters indicate significant differences ($p < 0.05$) among seasons for a given IWG stand age (2=upper-case; 3=lower-case). Within a given season, an asterisk indicates a significant difference in experimental forage yield between IWG stand ages. Points are calculated means and vertical bars represent 95% confidence intervals.

among the seasons ($p < 0.0001$). The same pattern was observed in the DU system in year 2 with the RFV of the spring experimental forage being 23 units greater than in the fall ($p < 0.0001$) and the fall experimental forage RFV being 36 units greater than the summer straw RFV ($p < 0.0001$). Conversely, in year 3 in the DU system, the RFV of the summer straw was 9 units greater than for the fall experimental forage ($p = 0.04$). The top ranking of spring experimental forage overall was likely driven by lower acid detergent fiber - which enhances forage digestibility - observed in year 3, particularly in the DU system ([Supplementary Materials S1](#)).

Like CP, the seasonal RFV varied by year. From year 2 to year 3, the fall experimental forage RFV declined by 42 units in the GP system ($p < 0.0001$) and by 35 units in the DU system ($p < 0.0001$). Spring experimental forage RFV in the GP system also decreased by 26 units from year 2 to year 3 ($p < 0.0001$). The summer straw RFV increased from year 2 to 3 in the GP system ($p < 0.0001$) and the DU system ($p < 0.001$) by 13 and 10 units, respectively. In general, the RFV for a given year and season tended to be similar among the two agronomic systems and the greatest RFV was observed in spring. These results provide partial evidence to support the hypothesis that spring and fall would have the greatest RFV but provide little evidence of greater RFV in the DU system compared to the GP system.

3.5 Farm enterprise budget

In agreement with hypothesis #5, the net return to the enterprise for the DU system was statistically similar to the GP system ($p=0.19$). Yet, the mean estimated net return to the enterprise for the DU system was $\$112 \text{ ha}^{-1} \text{ yr}^{-1}$ less than in the GP system but $\$347 \text{ ha}^{-1} \text{ yr}^{-1}$ greater than for the GR system ([Table 2](#)). The annual net return to enterprise ranged from $-\$855 \text{ ha}^{-1} \text{ yr}^{-1}$ in the establishment year to over $\$2,000 \text{ ha}^{-1} \text{ yr}^{-1}$ in the GP system in year 3 ([Figure 6](#)). Although the DU system did not produce a net return numerically as high as the GP system our statistical results support our hypothesis that the DU system would produce at least as great of net returns to the enterprise as the GP system.

Grain sales generated the most revenue in the GR, GP, and DU systems ([Table 2](#)). The lower farmer grain yields in the DU system in year 2 meant that less grain was sold and thus there was less grain revenue in this system. Despite the extra expense of $\$126 \text{ ha}^{-1} \text{ yr}^{-1}$ to harvest straw, straw sales in the GP and DU systems were more than enough to offset the cost. Straw sales increased the net return to the enterprise by $\$585 \text{ ha}^{-1} \text{ yr}^{-1}$ in the GP system and $\$411 \text{ ha}^{-1} \text{ yr}^{-1}$ in the DU system compared with the GR system, which did not market straw. As with grain sales, the lower farmer straw yield in the DU system in year 2 resulted in less revenue from straw sales in this system. After accounting for the additional expenses of fencing, water, and the grazing labor costs associated with forage production ($\$135 \text{ ha}^{-1} \text{ yr}^{-1}$), forage sales contributed a net benefit of $\$60 \text{ ha}^{-1} \text{ yr}^{-1}$ to the DU system. Grazing the forage compensated for the lower farmer grain and straw yields and produced a total revenue in the DU system that was $\$21 \text{ ha}^{-1} \text{ yr}^{-1}$ more than in the GP system, and $\$606 \text{ ha}^{-1} \text{ yr}^{-1}$ more than the GR system. Nonetheless, the GP system produced the greatest net return to the enterprise because it did not incur grazing expenses of

TABLE 2 The farmer’s 2018-2021 enterprise budgets summarized on a per hectare per year basis for three intermediate wheatgrass production systems: grain only, grain+straw, and dual use for grain+straw+forage.

Expenses	Grain-only	Grain +Straw	Dual use
	ha ⁻¹ yr ⁻¹		
Land cost (rent = \$24.20 ha ⁻¹)	\$432	\$432	\$432
Seed (12 kg ha ⁻¹ @ \$24.2 ha ⁻¹)	\$73	\$73	\$73
Planting, no-till (\$61.75 ha ⁻¹)	\$15	\$15	\$15
Fertilizer (37,854 liters liquid dairy manure)	\$78	\$78	\$78
Weed control	\$48	\$48	\$48
Fencing	\$0	\$0	\$31
Water	\$0	\$0	\$26
Grain harvest (\$136 ha ⁻¹)	\$102	\$102	\$102
Grain handling & storage (\$0.07 kg ⁻¹)	\$29	\$29	\$27
Straw harvest	\$0	\$126	\$126
Grazing cost, labor	\$0	\$0	\$78
Management cost	\$188	\$188	\$188
Total expense	\$965	\$1,091	\$1,224
Yields & Revenue			
Grain, kg total uncleaned	418	418	380
Grain sold (total value, 2019= \$2.20 kg ⁻¹ ; 2020, 2021 = \$3.30 kg ⁻¹)	\$1,227	\$1,227	\$1,146
Straw, kg total 83% dry matter, avg RFV = 80	0	5,323	4,474
Straw (total value, \$0.11 kg ⁻¹ as feed)	\$0	\$585	\$492
Grazed forage, kg total dry matter, avg RFV 106	0	0	1,298
Grazed forage (total value, \$0.15 kg ⁻¹ dry matter)	\$0	\$0	\$195
Total Revenue	\$1,227	\$1,812	\$1,833
Net Return to Enterprise	\$262	\$721	\$609

Cost, prices, and revenue values reflect those experienced by the farmer.

$\$135 \text{ ha}^{-1} \text{ yr}^{-1}$, and because farmer grain and straw yields in year 2 contributed to higher grain and straw revenues than the DU system.

4 Discussion

4.1 Summer experimental grain and straw yields

Perennial grains such as IWG provide year-round ground cover that promotes multiple environmental benefits in addition to

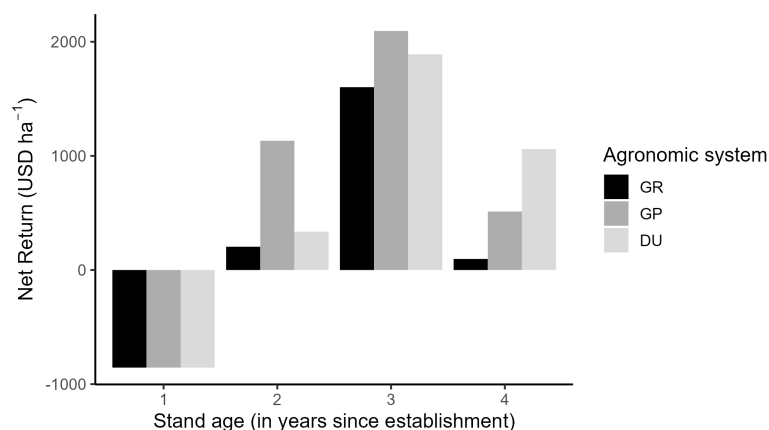


FIGURE 6

Farm enterprise data for an intermediate wheatgrass (IWG) grain-only production system (GR), grain+straw harvest (GP) system, and for dual use (DU) as a grain+straw and grazed forage crop. Bars indicate the actual total annual net return to enterprise for the establishment year (year 1) and three production years (2, 3, and 4).

producing a marketable grain. While gains in IWG grain yields have been made, this new crop yields (from 67% to 95.5%) less than comparable conventional annual wheat (Law et al., 2022; Culman et al., 2013). The latter has undergone millennia of informal selection and decades of formal crop improvement that contribute to current high yields. For IWG to be an attractive crop for growers to plant, the IWG system must offer opportunities to ensure profitability in spite of lower yields. It has been proposed that introducing livestock to graze the vegetative IWG growth between grain harvests can take advantage of on-farm resources during the off-season and generate an additional revenue stream (Zhen et al., 2024; Culman et al., 2023; Hunter et al., 2020a; Hunter et al., 2020b; Lanker et al., 2020).

In the present study, experimental grain yields in the DU system that were lower, the same, and greater than in the GP system depending on the year were observed. The effect of defoliation on IWG grain yields has previously been found to vary over time. For example, in the first year after establishing IWG, a spring defoliation event alone or with a fall defoliation event was associated with a greater grain yield than a defoliation event in the fall only, but two years later a spring defoliation event had the opposite effect (Hunter et al., 2020a). Compared with the undefoliated control, defoliation events in the spring+fall produced greater IWG grain yields in the second year after establishing IWG but after three years the grain yields in both treatments were the same, having both declined (Hunter et al., 2020a). A similar trend was observed in Ohio (Pugliese et al., 2019). Possible explanations for greater grain yields with defoliation include a reduction in lodging and an increased number of tillers m⁻¹ because of a reduced canopy following defoliation (Hunter et al., 2020b), neither of which were evaluated in the present study. Shorter plants are less vulnerable to lodging, increasing the harvestability of the crop. Defoliation, such as by haying and grazing, promotes greater light penetration through a reduced canopy which stimulates tiller recruitment (Da Silva et al., 2015). To limit the potential for reducing IWG grain yields in a DU system, attention must be paid to the timing of defoliation. For

example, defoliation in spring must occur before stem elongation to prevent removal of what will become the seed head to prevent reducing grain yield.

The decline of IWG grain yield with stand age that observed in the present study is well documented in the literature. For example, IWG breeders reported a 77% decline in grain yield after three years of production of the 'MN-Clearwater' IWG grain-type cultivar (Bajgain et al., 2020). The yields of a grain-type and a forage-type IWG declined 75 and 84%, respectively, from the first to the second year of production and further declines (48 and 35%) were observed from the second to third production season (Jungers et al., 2017). These declines exceeded the grain yield declines of 62% in the GP system and 25% in the DU system over the course of the present study. Researchers in Minnesota observed a ~50% decline in the number of IWG grains per spike after the first year of IWG grain production (Hunter et al., 2020a). The authors also reported the number of spikes producing grain declined with time, thus fewer spikes with less grain per spike likely led to lower IWG grain yield as the stand aged (Hunter et al., 2020a). Across nine North American sites, including a Minnesota site, yields appeared to be influenced by stand age more than by location, suggesting that yield may be more under genetic than environmental control (Culman et al., 2023). The authors noted greater IWG grain yield with greater annual precipitation and lower annual average temperatures, with the latter having a greater impact. In the present study, the greater IWG grain yields in years 1 and 2 coincided with average total annual precipitation that was greater than the 30-year average. Meanwhile, the average total annual precipitation was below average in year 3 and year 4 and IWG grain yields declined. Average annual temperatures were largely the same across years and similar to the 30-year averages in the present study, suggesting that precipitation may have had a larger role than temperature in this case.

Harvesting the IWG straw in addition to the grain added value to both the GP and DU systems. Experimental straw yields ranging from 4.99 Mg ha⁻¹ in the DU system to 9.21 Mg ha⁻¹ in the GP system were observed, which is within ranges previously reported in

the literature. For example, the average straw yields across nine North American sites were 5.21 and 6.47 Mg ha⁻¹ for two consecutive study years (Culman et al., 2023). In an organic IWG production system in New York, researchers reported straw yields of 5.73 Mg ha⁻¹ averaged over three years, which accounted for nearly half of the revenue generated from the sale of grain and straw (Law et al., 2021). Higher straw yields than what we observed have also been reported, such as results from five Minnesota locations that produced an average of 11.2 Mg ha⁻¹ for a grain-type IWG supplied with the agronomically optimum N rate (Jungers et al., 2017). As with IWG grain, we observed a decline in experimental straw yield in both systems as the IWG stand matured but whereas the GP system declined by 42% from one year to the next the DU declined by 8%. Similarly, previous research in a DU system reported a straw yield decline of 24% from the first to the second year of production (Hunter et al., 2020b). Experimental straw production in the DU system was less variable from year to year, although production was lower than in the GP system in the second year after establishing IWG.

The harvest index around 0.10 for IWG observed in the present study coincides with harvest index for IWG reported previously (Zhen et al., 2024) but it is lower compared with annual small grain crops like oat and barley. For example, oat and barley grown for grain in Italy had a harvest index of 0.35 and 0.45, respectively (Francia et al., 2006). The authors observed similar harvest index when the oat (0.36) and barley (0.41) were grazed once at the final tillering stage but lower harvest index when they were grazed a second time at the shoot elongation stage in oat (0.29) and the first node for barley (0.36). The relatively smaller grain size of IWG compared with conventional small grains, as well as the number of seeds per spike, and the proportion of fertile tillers (Hunter et al., 2020a) help to explain the lower harvest index in IWG. Increasing the IWG grain size remains a primary breeding goal for improving this novel crop (Bajgain et al., 2020).

4.2 Forage yield, herbage intake, forage utilization, and nutritive value

The fall regrowth and spring vegetative growth of IWG that was grazed by cattle in the present study produced experimental forage yields that reflect previously reported ranges. In a DU study in St. Paul, Minnesota the fall IWG hay yielded from 1.1 to 3.0 Mg ha⁻¹ while the spring IWG hay yielded from 1.0 to 2.4 Mg ha⁻¹, which together contributed to greater total forage (straw+hay) being observed in the DU system than in the control treatment where hay was not harvested (Hunter et al., 2020b). Summed together their fall and spring hay harvests yielded 3.5 to 4.0 Mg ha⁻¹ (Hunter et al., 2020b). Similarly, a study of two- and three-year old grain-type IWG stands harvested for hay either once (in summer), twice (in summer+September), or three times (in summer+September+November) yielded approximately 4 to nearly 5 Mg ha⁻¹ (Puka-Beals et al., 2022). These values are similar to the average fall+spring forage production of 4.0 Mg ha⁻¹ and 4.5 Mg ha⁻¹ observed for the DU and the GP systems, respectively.

Adequate herbage mass was available in both fall and spring to support grazing cattle in the DU system. A 60% HI was targeted, which was achieved for fall in two of three study years with the HI surpassing 90% in one of three years. Springtime HI, on the other hand, was generally closer to 40%. Spring HI values in the DU system are probably depressed since they would capture any leaf regrowth between the pre- and post-graze sample collection thereby reducing the difference between the two. Nonetheless, except for the fall of year 3, lower HI were observed than by researchers in Nebraska who grazed cattle at low [7,697 kg live weight (LW) ha⁻¹] and high (235,622 kg LW ha⁻¹) stocking densities, obtaining HI of 85 and 93%, respectively (Guretzky et al., 2020). Greater grazing pressure in the Nebraska study (i.e., more animals per unit area and a long grazing period in the high and low stocking density treatments, respectively) compared with the present study likely explains the differences in observed HI.

In terms of forage nutritive value, CP levels in spring experimental forage that remained relatively consistent across years were observed while fall experimental forage CP varied and summer experimental straw CP was consistently low. Puka-Beals et al. (2022) observed CP from 56 to 109 g kg⁻¹ for IWG biomass harvested once, twice or three times between July and November each year. This CP range coincides with what was observed in the present study for summer and fall IWG forage. Similarly, the CP of spring hay, fall hay, and summer straw averaged 234, 122, and 34 g kg⁻¹, respectively, in a IWG grain plus biomass coproducts system (Hunter et al., 2020b). These results reflect the pattern of greater CP in spring forage>fall forage>summer straw observed here.

Like CP, RfV followed the same seasonal pattern. Seasonal differences arising between the agronomic systems likely resulted from lower acid detergent fiber (ADF) in the DU system than in the GP system (Supplementary Materials S1). The lower ADF in the DU system probably occurred in spring because the fall grazing removed more mature forage, giving rise to new shoots in the spring. Meanwhile, without grazing in the GP system remnants of fall leaf tissue likely were still present and thus captured in the spring sampling event. The nutritive value of forage declines with maturity, as the proportion of cellulose, hemicellulose, and lignin increases in plant tissues (Moore and Jung, 2001). Similarly, it is well understood that forage yield increases with maturity. Thus, increased yield is negatively correlated with RfV. This relationship may help to explain why the RfV observed in year 2 was greater than in year 3, but the experimental forage yield in year 2 was less than in year 3. Spring grazing in the DU system may take advantage of forage with a greater RfV but has sometimes led to decreases in grain and straw yield (Hunter et al., 2020a). In the present study, it cannot be concluded whether the spring grazing in the DU system reduced the experimental grain and straw yields in one of three study years.

The RfV results reflect those reported in the literature. Culman et al. (2023) reported similar RfV values in the GP and DU systems they studied across nine temperate North American sites. The authors suggested focusing efforts to improve RfV in IWG on breeding rather than defoliation management since the most important factor influencing IWG RfV was season, which follows well-established trends (Culman et al., 2023). Previous research

identified a relationship between lower IWG RFV and an increasing number of growing degree days (GDD; Puka-Beals et al., 2022), which accumulate more rapidly with warmer temperatures. Hunter et al. (2020b) reported fewer GDD accumulated in the spring (470°C d) than in the fall (1,280°C d) and in the summer (2,200°C d) which likely explained the seasonal pattern of RFV that they observed in IWG RFV. A Wisconsin study of an IWG monoculture and an intercrop of IWG+red clover (*Trifolium pratense* L.) also reported RFV values of 175, 116, and 65 for spring, fall, and summer, respectively, in the IWG monoculture (Favre et al., 2019). While their values for fall and spring forage were greater than those observed here (101 and 127), higher summer straw RFV (75 to 85 vs 65) were observed in the present study. The RFV is used as an indicator for determining the price paid for straw and forage. The sale of the abundant, low quality summer straw and of good quality forage, albeit of limited volume, factors into the profitability of IWG DU systems.

4.3 Farm enterprise budget

Finally, GP and DU systems were profitable but the greatest net profit was for the GP system that included a summer grain as well as a straw harvest and sales. On the basis of net return to the enterprise, a value of \$1,247 ha⁻¹ yr⁻¹ for the GP system and \$1,096 ha⁻¹ yr⁻¹ for the DU was reported while in New York an organically-managed IWG crop that produced grain+straw generated a mean annual income of \$432.93 ha⁻¹ yr⁻¹ (Law et al., 2022). The production expenses were lower in the New York study but the revenues were too. Although the present study observed that the GP system had the greatest numeric net return, the DU system was also profitable, suggesting that producers with livestock can benefit from grazing their cattle on IWG in the spring and fall when feed supply is low. The present study did not reflect the feed costs offset by grazing IWG, nor the animal gain which influences the sale price of cows. These budget items would provide additional insight into the costs and benefits of an IWG DU system.

Consideration of management decisions such as when to graze cattle on IWG can impact profitability. For example, a Minnesota study reported a DU system for grain and hay with a single fall harvest more consistently produced the best net returns compared with hay harvested in the spring only or in the fall+spring (Hunter et al., 2020b). Similarly, researchers reported diminishing returns from a third fall hay harvest compared with a single or two hay harvests per season in mature IWG stands due to limited vegetative growth between September and November (Puka-Beals et al., 2022). Thus, consideration must be given to ensure that the economic value of grazing offsets the costs of each additional grazing event.

This study was conducted in a region where row crop agriculture is economically competitive with livestock production because of the favorable climate and highly productive soils in the region. When IWG is grown on marginal land in the region, though, the DU benefit of IWG may increase since the crop can yield grain, straw, and forage on land deemed unsuitable for row crop production thereby generating up to three marketable

products or at least reducing input costs of cattle feed. Moreover, as a drought tolerant species, IWG has significant potential to function as a profitable grain crop in more arid regions including the Great Plains and Intermountain West in the US. In these areas, the lower yield potential of row crops and relatively lower land prices could increase the profitability of DU IWG production and studies like this should be conducted in regions with varying growing conditions and access to agricultural markets.

Other directions for future research might compare harvesting hay vs. grazing cattle since mowing could reduce some of the fixed costs associated with managing animals (e.g., water, fencing) in a DU system. However, the costs of additional mechanical harvest and labor needs will need to be considered for a hay operation. Beyond costs, comparing these two systems may elucidate which approach, cutting hay vs. grazing, may be more feasible under different production conditions, such as years with greater or lower IWG biomass production. Studies of the impacts of incorporating cattle into IWG cropping systems on ecosystem services, such as N cycling and C storage, could highlight potential tradeoffs beyond yield and forage quality that might result from grazing IWG in a DU system. Lastly, as new cultivars of grain-type IWG come onto the market, evaluating their potential for a DU system can increase the management options available for IWG producers.

5 Conclusion

This study compared the productivity and profitability of two IWG agronomic systems. A DU system that utilized IWG for forage as well as grain and straw production extended the growing season into the fall and spring, allowing for multiple biomass harvests within a single growing season to maximize the agronomic productivity of the land, and solar energy and precipitation utilization. Furthermore, the DU system generated an additional revenue stream by valorizing forage production, which led to increased profitability compared with grain sales alone. For both grain+straw (GP) and DU systems, harvesting summer straw is recommended to increase net returns to the enterprise. Although the DU system had a lower net return than the GP system due to lower grain and straw yields in one of three years, these findings underscore the potential of a DU system to particularly enhance the productivity of more mature IWG cropping stands to generate additional income at a time when IWG grain yields decline. Where livestock are already present in an operation, grazing IWG in spring and fall can supply forage of good nutritive value to help offset the costs of purchasing feed during periods of limited forage availability. Overall, these results demonstrate how a perennial grain crop can achieve goals of sustainable intensification and provides a model that could facilitate an agroecological transition in the short-term.

Data availability statement

The code and data sets supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

HR: Conceptualization, Data curation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. MH: Data curation, Investigation, Methodology, Writing – review & editing. AK: Data curation, Investigation, Methodology, Writing – review & editing. NT: Funding acquisition, Writing – review & editing. JJ: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

Funding

The author(s) declare that financial support was received for the research and/or publication of this article. Funding for this project was provided by the Minnesota Department of Agriculture Sustainable Agriculture Demonstration Grant Program (SWIFT Contract #138801; PO #3000027068).

Acknowledgments

The authors would like to thank Jesse Puka-Beals, Dane McKittrick, Loren Dauer, Katherine Bohn, and the rest of the Sustainable Cropping Systems Lab staff for their dedication and

assistance with essential research activities. We would also like to thank Kaleb Anderson for his invaluable contributions to data collection and site maintenance.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fagro.2025.1534962/full#supplementary-material>

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OPEN ACCESS

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RECEIVED 24 December 2024

ACCEPTED 09 April 2025

PUBLISHED 08 May 2025

CITATION

Scavo A, Maio A, Calderone F, La Malfa T,
Trostle C, Yates RJ, Toscano S, Cavallo C,
Oteri M, Furfaro ME, Virga AN, Scordia D and
Gresta F (2025) Enhancing Mediterranean
forage legume production through
inoculation with elite rhizobia.
Front. Agron. 7:1551176.
doi: 10.3389/fagro.2025.1551176

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Enhancing Mediterranean forage legume production through inoculation with elite rhizobia

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Forage legumes play a fundamental role in the sustainability of cropping systems, as rotating species with grain crops, intercrops, or winter cover crops. However, their compatibility with rhizobial inoculants needs context-specific studies. The objectives were to evaluate the effectiveness of three species-specific inoculants [Australian granular (AUG), Australian peat (AUP), and American peat (USP)], compared with a non-inoculated control (CNT). These were applied at the recommended and double dose on five Mediterranean forage legumes (*Vicia sativa*, *Medicago polymorpha*, *Trifolium michelianum*, *T. subterraneum*, and *T. pratense*). Plant growth, nodulation, and relative N₂ fixation were measured. Species-specific variations were observed for each inoculant. Across the average of legume species, AUG demonstrated the highest growth- and nodulation-promoting effects at both standard and double inoculum doses. The USP was the worst inoculant at the standard dose but induced positive effects at double dose. The relative N₂ fixation was only improved at double dose, especially by USP and AUG, whereas only AUP provided significant N₂ fixation enhancements at standard dose. Overall, the double dose was the best strategy for all tested forage legumes. These findings suggest that inoculating Mediterranean forage legumes with selected inoculants, especially at double dose, may be an effective solution to increase their N₂ fixation ability, reduce the use of mineral N fertilizers, and identify the optimal forage legume × inoculant combinations for intercropping systems with cereals.

KEYWORDS

agroecology, *Rhizobium*, biological nitrogen fixation, plant growth, nodulation, sustainability

1 Introduction

Agroecosystems are facing a significant decline in soil fertility due to the intensification of agricultural practices and climate change. This is especially true in semi-arid regions where erratic rainfall, frequent soil erosion events, increased salinity, and severe weed pressure are characteristic features (Abdelhak, 2022). Under these conditions of low soil organic matter, nitrogen (N) is generally the most limiting soil macronutrient. It is estimated applied N for plant growth is effectively incorporated into agricultural products at a rate of 40%–50%. While the remaining is subjected to losses by nitrate leaching (NO₃), ammonia volatilization (NH₃), and nitrous oxide (N₂O) emissions, causing environmental burdens (Mahmud et al., 2021). The environmental impact, coupled with increasing costs of mineral N fertilizers, demands the scouting for alternative sources of N (Allito et al., 2020).

The symbiotic biological N fixation (BNF) by N-fixing bacteria, especially those of the family *Rhizobiaceae*, is a sustainable and alternative source of available N. The bacteria allow reduced N mineral fertilizer application while maintaining high crop yields, in agreement with the European *Green Deal* and the United Nations (UN) Sustainable Development Goals (United Nation (UN), 2015; European Commission (EC), 2019). Effective root nodule symbiosis not only allows legumes to grow in N-poor soils but also increases the soil N levels for the subsequent rotational cash crops or for companion crops in polycultural systems (Drevon et al., 2015; Schwember et al., 2019; Scordia et al., 2024). However, BNF is species specific, and its efficiency depends on legume genotype and bacterial strain (Allito et al., 2021; Kohlmeier et al., 2023). Soil bacteria associated with legumes for BNF are commonly termed rhizobia. The family *Rhizobiaceae* includes at least 168 species in 17 genera, of which those with the highest number of described species are *Rhizobium*, *Bradyrhizobium*, *Mesorhizobium*, and *Sinorhizobium* (Kuzmanović et al., 2022). Rhizobial strains are supplied through legume inoculants, crop species-specific products containing isolates of live rhizobia protected by organic carrier material (Lupwayi et al., 2000). Although large-scale production of legume inoculants is complex, the legume inoculant industry is now well established (O'Callaghan et al., 2022), with several different commercial inoculant formulations such as peat, granular, liquid, and freeze-dried powders (Howieson and Dilworth, 2016). Inoculant quality depends on the cell numbers of a selected rhizobial strain, an easy-to-apply and effective formulation, an adequate shelf life, and the dose of application (Lupwayi et al., 2000). Nonetheless, it has been demonstrated that applying inoculants at higher than recommended rates could be beneficial, especially where forage legumes are grown in adverse soil conditions such as low pH (Farquharson et al., 2022; Frame and Laidlaw, 2005).

Forage legumes are a key component for the sustainability of pastures and are key to livestock production, BNF, soil organic matter levels, and soil erosion mitigation (Sheaffer and Seguin, 2003). They are commonly cultivated in rotation with grain crops, but in recent years, they also emerged as intercrops or winter cover crops (Holman et al., 2018; Vujić et al., 2021; Scordia et al., 2024).

Carlsson and Huss-Danell (2003), who investigated the N₂ fixation in three perennial forage legumes primarily relating to ungrazed northern temperate/boreal areas, reported BNF rates up to 545 kg N ha⁻¹ year⁻¹ in white clover (*Trifolium repens*), 350 kg N ha⁻¹ year⁻¹ in alfalfa (*Medicago sativa* L.), and 373 kg N ha⁻¹ year⁻¹ in red clover (*T. pratense*). In Australia, it is estimated forage legumes fix 3.5 million tonnes of N annually on about 45 million hectares, equivalent to a national value of up to Aus\$ 3.5 billion annually (Farquharson et al., 2022). Forage legumes are a traditional component of Mediterranean grassland communities such as Syria, Greece, Sardinia, Sicily, Morocco, and Tunisia, coevolving over the last 1,000 years with native rhizobial populations (Howieson et al., 2000). Unfortunately, the high soil temperatures of arid and semi-arid Mediterranean regions, coupled with severe salinity levels and desertification processes that are increasing under the climate change effects, may affect negatively forage legume-rhizobia associations (Rejili et al., 2012). Furthermore, most farmers of legumes in the Mediterranean region and elsewhere assume that their fields nodulate and fix nitrogen as they have not been adequately trained; thus, they have never examined the roots for active nodulation. This, too, presents a risk to productive farming if legumes are undernodulated. Hence, there is a need for legume inoculation with elite rhizobia to provide and to ensure optimal BNF, which in turn is required to offset increasing agronomic challenges. These include farming practices such as monoculture plantings, low-frequency (over 5 years) legume rotational break crops, soil acidification, detrimental residual soil herbicides, lack of certainty in field nodulation, and uncertain climatic conditions (Yates et al., 2024). However, the compatibility of new forage legume species or cultivars to agriculture with commercial rhizobial inoculants requires ongoing research support to optimise the symbiosis (Rigg et al., 2021; Shi et al., 2023).

In this preliminary work, considering the increasing diffusion of cereal-legume double cropping, five forage legumes common to Mediterranean regions (*Vicia sativa* L., *M. polymorpha*, *Trifolium michelianum* Savi, *T. subterraneum*, and *T. pratense*) were screened in pot bioassays under edaphic and climatic uncontrolled conditions to select the optimal inoculant formulation and dose of application for each legume-rhizobial strain combination for testing in future intercropping systems. These forage legumes exhibit different rhizobial associations and are highly specific to the micro-symbiont they nodulate to achieve an effective symbiosis (Kohlmeier et al., 2025). For instance, *V. sativa* forms a symbiosis with the micro-symbiont *Rhizobium leguminosarum* bv. *viciae*, although differences in host plant preference for specific rhizobial genotypes within natural populations have been observed (Laguerre et al., 2003). Clovers and medics are nodulated and can form an effective symbiosis with *R. leguminosarum* biovar *trifolii* and *Sinorhizobium* spp., respectively (Charman and Ballard, 2004; Farquharson et al., 2022). Our goals were (1) to evaluate the effect of inoculant formulations and rhizobial strains on plant growth, nodulation, and N₂ fixation of selected forage legumes, (2) to assess whether increasing the inoculant rate could further improve the dependent variables, and (3) whether the phenological growth stage could affect the inoculation efficiency.

2 Materials and methods

2.1 Experimental design

Two different pot trials under natural conditions, hereafter referred to as Experiments A and B, were set up in 2022/2023 in a private farm located in Milazzo (Messina, 38°11'25" N, 15°14'28" E) according to a complete randomized block design with three replications. In Experiment A, four forage legumes were inoculated with three species-specific different inoculants (an Australian granular, AUG; an Australian peat, AUP; an American peat from Visjon Exceed®, USP), versus a non-inoculated control (CNT). Inoculants were seed applied at the recommended application method and dose at sowing, and crops were harvested at the vegetative growth stage (just prior to reproductive growth). In Experiment B, five forage legumes were inoculated with the same Experiment A inoculants but at double dose, and crops were harvested in the reproductive growth stage to respectively assess the second and third research objectives.

2.2 Plant material

The forage legumes employed in Experiment A were *Vicia sativa* L. var. Buza, *M. polymorpha* var. Scimitar, *Trifolium michelianum* Savi (local ecotype), and *T. subterraneum* var. Urana. In Experiment B, *T. pratense* var. Rozeta was added. *M. polymorpha*, *T. subterraneum*, and *T. michelianum*, annual self-seeding species with autumn-winter-spring cycle (Charman and Ballard, 2004; Scavo et al., 2021), were purchased from Padana sementi (Tombolo, Padua, Italy). *V. sativa* and *T. pratense* were two local ecotypes. Although the legume species investigated in the present study differ in growth/biomass production potentials, growth habits, and life cycle length due to inherent genetic differences, they are adapted to semiarid environments and were selected based on predominant winter growth in Mediterranean climates (Blackwell et al., 2018; Enriquez-Hidalgo et al., 2020; Scavo et al., 2021).

2.3 Pot trials

Experiments were conducted in dark plastic pots (diameter 20 cm; height 20 cm; volume 5 L) filled with a substrate composed

of 3 cm of stones at the base to prevent soil saturation, soil from the transition zone between natural pastures and forest in Messina mountains, and peat (Pindstrup Mosebrug A/S, Denmark). The substrate components are described in Table 1. Ten seeds pot⁻¹ for each species were sown at 1 cm depth and then thinned to ensure a homogeneous population density of three plants pot⁻¹. Detailed information on sowing, thinning, and harvest dates is shown in Table 2. Starting from sowing, pots were drip irrigated every 2 days for a total of 12,600 ml pot⁻¹ in Experiment A and 9,400 ml pot⁻¹ in Experiment B. No fertilization or pest control treatments were applied.

Inoculation was carried out at sowing on 21 August 2023 for Experiment A and 7 September 2023 for Experiment B following the recommendations reported in the product labels. In detail, AUG and AUP inoculants were kindly provided by Murdoch University (Australia). AUG and AUP specific strains were *Rhizobium leguminosarum* bv. viciae (Australian Group E – WSM4643) for common vetch, *Sinorhizobium* spp. (Australian Group AM – WSM1115) for burr medic and *Rhizobium leguminosarum* bv. trifolii (Australian Group C – WSM1325) for clovers. USP

TABLE 1 Main physico-chemical characteristics of the soil at the beginning of Experiments A and B.

Soil characteristic	Unit of measurement	Value
Sand	%	40
Clay	%	40
Silt	%	20
Organic matter	g kg ⁻¹	4.9
Total CaCO ₃	g kg ⁻¹	9
Total N	g kg ⁻¹	1.5
Available P	mg kg ⁻¹	86
pH		7.6
Electrical conductivity	mS cm ⁻¹	0.18
Cation exchange capacity	meq 100 g ⁻¹	4.7
CaO	mg kg ⁻¹	772
MgO	mg kg ⁻¹	27
Extractable K	mg kg ⁻¹	21

TABLE 2 Sowing, thinning and harvest dates, and length of the biological cycle expressed as days after sowing (DAS) for each legume species in Experiments A and B.

Forage legume species	Experiment A				Experiment B			
	Sowing	Thinning	Harvest	DAS	Sowing	Thinning	Harvest	DAS
<i>Vicia sativa</i>	21/08/2023	18/09/2023	20/12/2023	121	07/09/2023	26/09/2023	26/03/2024	201
<i>Medicago polymorpha</i>	21/08/2023	14/09/2023	20/12/2023	121	07/09/2023	06/10/2023	09/04/2024	215
<i>Trifolium michelianum</i>	21/08/2023	14/09/2023	27/12/2023	128	07/09/2023	06/10/2023	23/03/2024	201
<i>Trifolium subterraneum</i>	21/08/2023	14/09/2023	27/12/2023	128	07/09/2023	06/10/2023	09/04/2024	215
<i>Trifolium pratense</i>					07/09/2023	06/10/2023	15/04/2024	221

consisted of three species-specific inoculants containing either *Rhizobium leguminosarum* bv. viciae or bv. trifolii depending on the legume species. Australian inoculants at standard doses (Experiment A) were inoculated as slurry to coat the seed by mixing 1.5 g (for AUP) or 2.5 g (for AUG) of each inoculant strain with 30 ml of water and injecting it into a moist seedbed (25 g of peat substrate with 500 ml of water) at sowing. For double doses (Experiment B), double inoculant rates were applied in the same amount of water as the standard rate. The USP was seed applied by mixing 0.75 g of inoculum on 100 g of seeds for each forage legume

previously soaked with 1.1 ml of water. Detailed information about inoculants is summarized in Table 3.

Weather conditions were obtained from a weather station of the SIAS (Servizio Informativo Agrometeorologico Siciliano, www.sias.regione.sicilia.it), close to the experimental site. Total rainfall and reference evapotranspiration (ET_0) during the experimental period (August–April) were 469 and 780.2 mm, respectively (Table 4). Except for the November–February period, the remaining months experienced water deficits, especially August (−149 mm) and October (−97 mm). In general, weather conditions

TABLE 3 Description of the inoculant treatments adopted in Experiments A and B pot trials.

Inoculant formulation and source	Treatment code	<i>Rhizobium</i> inoculum	Forage legume	Inoculum potential	Application rate (g pot ^{−1})
Australian granular (Murdoch Univ.)	AUG.1	<i>Rhizobium leguminosarum</i> bv. viciae	<i>Vicia sativa</i> var. Buza	(1 × 10 ⁹ CFU g ^{−1})	2.5 (Exp. A) and 5.0 (Exp. B)
	AUG.2	<i>R. leguminosarum</i> bv. trifolii	<i>Trifolium michelianum</i> , <i>T. subterraneum</i> var. Urana, † <i>T. pratense</i> var. Rozeta	(1 × 10 ⁹ CFU g ^{−1})	2.5 (Exp. A) and 5.0 (Exp. B)
	AUG.3	<i>Sinorhizobium</i> spp.	<i>Medicago polymorpha</i> var. Scimitar	(1 × 10 ⁹ CFU g ^{−1})	2.5 (Exp. A) and 5.0 (Exp. B)
Australian peat (Murdoch Univ.)	AUP.1	<i>R. leguminosarum</i> bv. viciae	<i>V. sativa</i>	(1 × 10 ⁹ CFU g ^{−1})	1.5 (Exp. A) and 3.0 (Exp. B)
	AUP.2	<i>R. leguminosarum</i> bv. trifolii	<i>T. michelianum</i> , <i>T. subterraneum</i> , <i>T. Pratense</i>	(1 × 10 ⁹ CFU g ^{−1})	1.5 (Exp. A) and 3.0 (Exp. B)
	AUP.3	<i>Sinorhizobium</i> spp.	<i>M. polymorpha</i>	(1 × 10 ⁹ CFU g ^{−1})	1.5 (Exp. A) and 3.0 (Exp. B)
American peat (Visjon Biologics)	USP.1	Exceed® Pea/Vetch/Lentil: <i>R. leguminosarum</i> bv. viciae	<i>V. sativa</i>	(2 × 10 ⁸ CFU g ^{−1})	1.5 (Exp. A) and 3.0 (Exp. B)
	USP.2	Exceed® Subterranean Clover: <i>R. leguminosarum</i> bv. trifolii (subterranean)	<i>T. subterraneum</i>	(2 × 10 ⁸ CFU g ^{−1})	1.5 (Exp. A) and 3.0 (Exp. B)
	USP.3	Exceed® True Clover: <i>S. meliloti</i> and <i>R. leguminosarum</i> bv. trifolii	‡ <i>M. polymorpha</i> , <i>T. michelianum</i> , <i>T. Pratense</i>	(2 × 10 ⁸ CFU g ^{−1})	1.5 (Exp. A) and 3.0 (Exp. B)
Non-inoculated control	CNT	None	All	Unknown	None

†*T. pratense* in Exp. B only. ‡Visjon Biologics does not market a crop-specific inoculant for *M. polymorpha*, *T. michelianum* and *T. pratense*, but suggested trying Exceed “Alfalfa/True Clover”, a blend of *S. meliloti* and *R. leguminosarum* bv. trifolii (clover).

TABLE 4 Weather conditions [maximum and minimum temperatures, mean relative humidity (RH), rainfall, and reference evapotranspiration (ET_0)] at the experimental site (Milazzo, 38°11'25" N, 15°14'28" E) during the 2023/2024 growing seasons of Experiments A and B.

	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr
T _{max} (°C)	31.0	29.9	28.4	22.8	18.5	17.2	17.7	19.8	22.3
T _{min} (°C)	20.4	19.1	17.5	12.9	9.8	8.0	8.6	9.9	10.4
RH _{mean} (%)	53.1	53.4	57.1	60.6	63.3	64.2	63.4	60.6	53.5
Rainfall (mm)	14.0	32.8	1.2	77.6	63.8	77.0	121.2	55.6	25.8
ET ₀ (mm)	162.9	119.3	98.4	59.9	41.2	44.1	54.8	88.8	110.9

were optimal for the growth of forage legumes and supplemented with drip irrigation when required.

2.4 Measurements

After harvest (Table 1), soil substrates were removed from pots, the roots were gently washed with tap water, and the aboveground and belowground plant parts were carefully separated. Aboveground and belowground fresh weight, root nodulation (nodule counts), and total nodule fresh weight were measured on the three plants pot⁻¹. Dry matter was determined by oven-drying biomass samples at 65°C up to constant weight.

Aboveground and belowground samples were ground to pass a 1-mm sieve (CyclotecTM 1093 Sample Mill, Foss, Denmark), and the total nitrogen was determined according to the Kjeldahl method (UDK 169, Velp Scientifica, Italy).

2.5 Estimation of relative N₂ fixation

N₂ fixation was estimated by the N-difference method, in which the N yields of fixing plants are compared with an uninoculated legume of the same species (control). This is a simple and low-cost method based on the arbitrary assumption that plants absorb the same amount of N from the soil and translocate equal amounts of soil-derived N (Hardarson and Danso, 1993). Here, we adapted the method by considering the uninoculated legumes of the same species as control plants, aware that uninoculated control plants can nodulate due to the presence of native rhizobial communities in the experimental soils. We did not use sterile soil in control pots because it would have changed the soil microbiome and thus the inoculation efficiency, given that they act in synergism with plant-growth-promoting rhizobacteria (Tilak et al., 2006). N yields of aboveground (ANY) and belowground (BNY) plant parts were calculated as:

$$\text{ANY} = (\text{aboveground DW}) \times (\% \text{N}_{\text{shoots}})$$

$$\text{BNY} = (\text{belowground DW}) \times (\% \text{N}_{\text{roots}})$$

where %N_{shoots} and %N_{roots} are the total nitrogen in above and belowground of legume species, respectively. The amount of relative

N₂ fixation was quantified in accordance with the equation proposed by Howieson and Dilworth (2016):

$$\text{Nfixed} = (\text{total N yield inoculated}) \\ - (\text{total N yield non-inoculated control})$$

where total N yield is the sum of aboveground and belowground N yields. Given that inoculated and non-inoculated plants grew with the same pedo-climatic conditions and management, we assume that the difference in N fixed could be attributable to the inoculation effect.

2.6 Statistical analysis

Data for each experiment were analyzed separately by two-way analysis of variance (ANOVA), according to the complete randomized design. The legume species and the inoculant type (rhizobial strain × inoculant formulation combination) were the fixed effects. Before the ANOVA, the homogeneity of variance was assessed with the Bartlett's test and normality by a graphical inspection of the residuals. Percentage data were arcsine √% transformed before the analysis. The significance of differences among groups was tested using the Tukey's HSD test at $p \leq 0.05$. All analyses were conducted using CoStat[®] software version 6.003 (Cohort Software, Monterey, CA, USA).

3 Results

3.1 Experiment A

3.1.1 Plant growth and nodulation

The ANOVA showed that legume species contributed the most to the overall variance for all the investigated parameters (Table 5). The effect of inoculant formulation was significant for root nodulation and total nodule weight, whereas aboveground and belowground biomass were significantly affected only by legume species. Moreover, a significant 'species × inoculant' effect was found for belowground biomass and root nodulation.

The effects on aboveground and belowground biomass, which were the highest in *T. subterraneum* (17.2 and 16.6 g pot⁻¹, respectively) and *T. michelianum* (13.0 and 15.4 g pot⁻¹), were

TABLE 5 Analysis of variance (ANOVA) according to the complete randomized design for main factors and their interactions in Experiment A for aboveground dry weight (ADW), belowground dry weight (BDW), root nodulation (RN), total nodule weight (NW), aboveground nitrogen yield (ANY), belowground nitrogen yield (BNY) and relative N₂ fixation.

Source	df	ADW	BDW	RN	NW	ANY	BNY	Relative N ₂ fixation
Species (S)	3	346.01 ***	602.66 ***	238884.74 ***	1.21 ***	1938.3 ***	1029.5 ***	717.2 ***
Inoculant (I)	3	21.88 ns	13.95 ns	53613.73 ***	0.74 **	167.9 ns	11.6 ns	330.7 ***
(S) × (I)	9	8.46 ns	21.97 **	52824.78 ***	0.14 ns	67.6 ns	26.7 **	121.4 ***
Error	30	8.94	6.53	1292.36	0.10	65.1	8.5	18.8

Values are given as mean square (MS); df, degrees of freedom; *** and ** indicate significance at $p \leq 0.001$ and $p \leq 0.01$, respectively (Tukey's HSD test); ns, not significant.

species specific (Figure 1). In fact, all inoculants investigated decreased belowground biomass of *T. subterraneum* and *T. michelianum* compared with the control (CNT), while an opposite trend was observed for *V. sativa*. In *M. polymorpha*, except for USP that reduced belowground biomass, AUG and AUP showed not significant differences compared to CNT.

Root nodulation was significantly improved by AUG (+60% than control), followed by AUP (+23.6%) and USP (+11.5%) (Figure 1). The greatest root nodulation was found in *V. sativa* and *T. subterraneum* inoculated with AUG (555 and 547 number pot^{-1} , respectively). AUP induced the highest root nodulation in *T. michelianum* (445 number pot^{-1}), while no positive effect was observed in *M. polymorpha*.

Total nodule fresh weight showed a similar trend to root nodulation (Figure 1). AUG determined the highest total nodule weight (1.1 g pot^{-1}), while both AUP and USP caused lower values than CNT. Across the average of inoculants, no significant differences were observed between *V. sativa*, *T. michelianum*, and

T. subterraneum, while *M. polymorpha* had the lowest total nodule weight (0.3 g pot^{-1}).

3.1.2 Relative N_2 fixation

From the ANOVA emerged that the “species $\times$ inoculant” interaction was significant on BNY and N_2 fixation (Table 5). ANY, BNY, and relative N_2 fixation were mostly affected by legume species, and the inoculant effect was not significant for ANY and BNY.

Across the average of inoculants, *T. subterraneum* and *T. michelianum* showed the highest ANY (43.5 and 33.0 mg N pot^{-1} , respectively) and BNY (24.9 and 18.2 mg N pot^{-1}), while no significant effects were observed between the inoculants (Figure 2). Concerning BNY, the two-way interaction showed that it was increased by all inoculants in *V. sativa* and decreased in *T. michelianum*, although without significant differences. No significant differences among the inoculants were also detected in *T. subterraneum*. USP was the worst inoculant for all forage legumes.

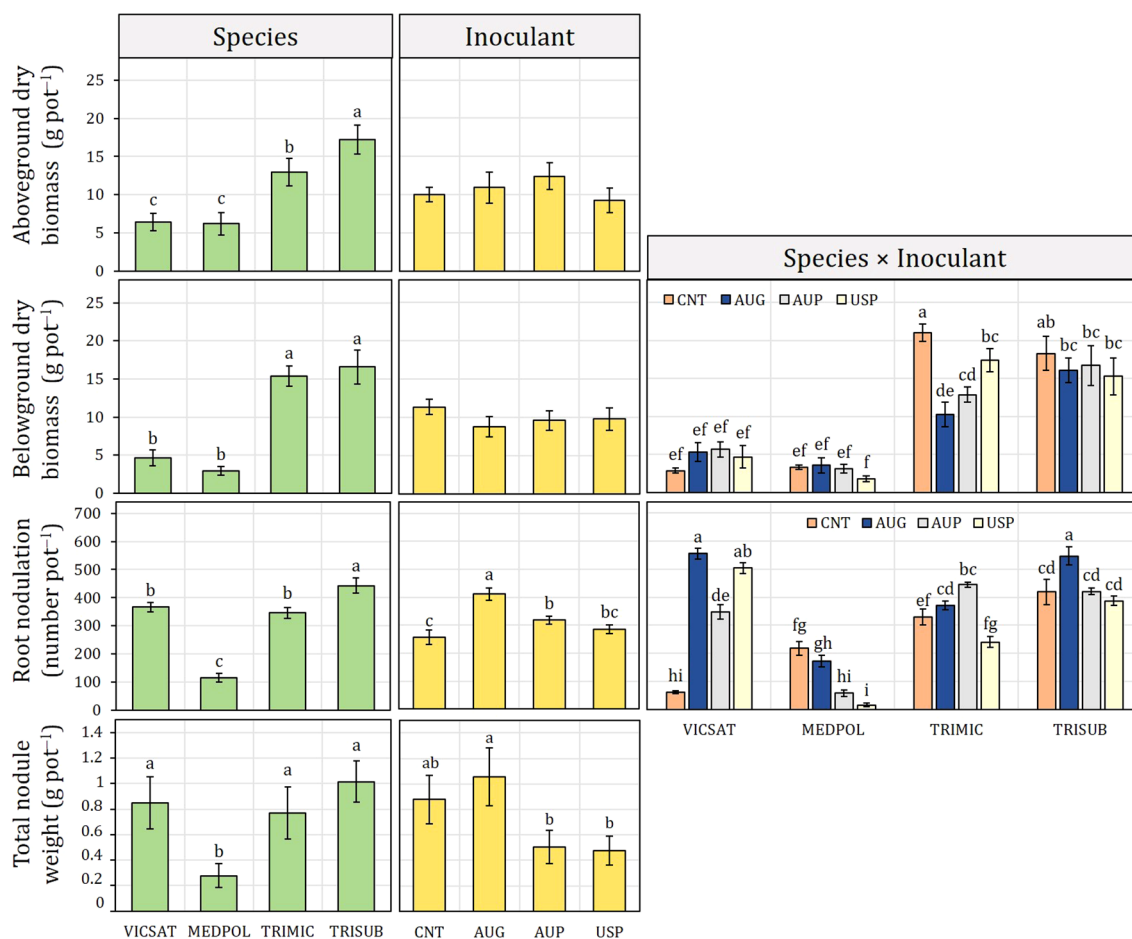


FIGURE 1

Inoculant formulation effect (non-inoculated control, CNT; Australian granular, AUG; Australian peat, AUP; American peat from Visjon Exceed[®], USP) on aboveground and belowground dry biomass, root nodulation, and total nodule fresh weight of *Vicia sativa* (VICSAT), *Medicago polymorpha* (MEDPOL), *Trifolium michelianum* (TRIMIC), and *Trifolium subterraneum* (TRISUB) from Experiment A. Bars are standard error. Different letters indicate statistical significance at $p \leq 0.05$ (Tukey's HSD test).

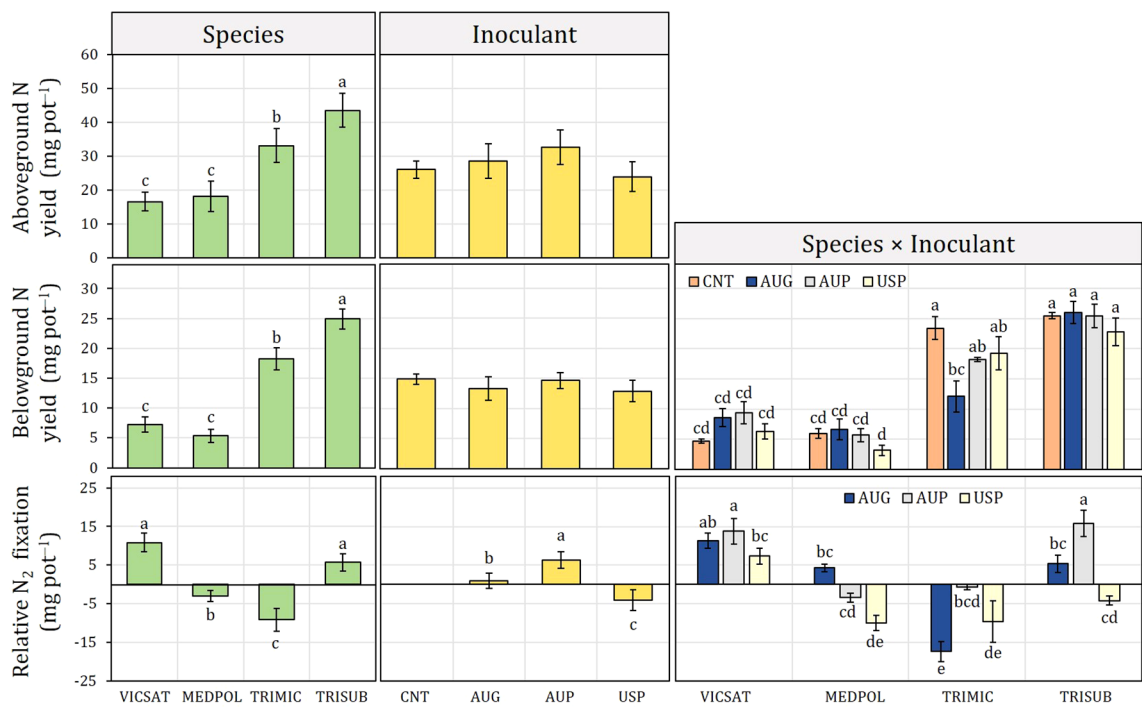


FIGURE 2
Inoculant formulation effect (non-inoculated control: CNT; Australian granular: AUG; Australian peat, AUP; American peat from Visjon Exceed®, USP) on aboveground N yield, belowground N yield and relative N₂ fixation of *Vicia sativa* (VIC SAT), *Medicago polymorpha* (MED POL), *Trifolium michelianum* (TRIM IC), and *Trifolium subterraneum* (TRISUB) from Experiment A. Bars are standard error. Different letters indicate statistical significance at $p \leq 0.05$ (Tukey's HSD test).

Across the average of species, AUP induced the greatest relative N₂ fixation (6.4 mg N pot⁻¹), followed by AUG (0.8 mg N pot⁻¹), whereas USP fixed less than CNT (Figure 2). *V. sativa* was the best fixing species among the forage legumes investigated (10.8 mg N pot⁻¹), followed by *T. subterraneum* (5.7 mg N pot⁻¹), while *M. polymorpha* and *T. michelianum* fixed less than CNT with the investigated inoculants at the standard application rate. In detail, the inoculants improved the relative N₂ fixation in *V. sativa* and *T. subterraneum*, excluding USP for the latter species, with AUP that determined the highest values for both species (13.7 and 15.7 mg N pot⁻¹, respectively). Only AUG induced relative N₂ fixation in *M. polymorpha* (4.3 mg N pot⁻¹), while none of the inoculants was effective in *T. michelianum*.

3.2 Experiment B

3.2.1 Plant growth and nodulation

Except for the aboveground biomass, the ANOVA highlighted a significant “species × inoculant” effect for all the investigated parameters (Table 6). Legume species were confirmed as the major factor affecting variance.

Across the average of species, USP had the highest stimulating effect on aboveground biomass (23.3 g pot⁻¹, +63.8% than CNT), followed by AUG (19.5 g pot⁻¹) (Figure 3). Concerning belowground biomass, no significant differences were detected between inoculants and the control for *V. sativa*. The highest belowground biomass for *M. polymorpha* was induced by USP

TABLE 6 Analysis of variance (ANOVA) according to the complete randomized design for main factors and their interactions in Experiment B for aboveground dry weight (ADW), belowground dry weight (BDW), root nodulation (RN), total nodule weight (NW), aboveground nitrogen yield (ANY), belowground nitrogen yield (BNY), and relative N₂ fixation.

Source	df	ADW	BDW	RN	NW	ANY	BNY	Relative N ₂ fixation
Species (S)	4	1252.32 ***	381.45 ***	258531.50 ***	5.00 ***	5740.8 ***	2118.9 ***	393.7 **
Inoculant (I)	3	227.13 ***	65.54 ***	48700.21 ***	1.19 ***	906.7 ***	340.2 ***	404.7 *
(S) × (I)	12	56.28 ns	58.20 ***	29260.87 ***	0.64 ***	353.9 **	235.1 ***	734.9 ***
Error	38	32.71	5.70	2778.34	0.12	124.3	28.1	80.7

Values are given as mean square (MS); df, degrees of freedom; ***, **, and * indicate significance at $p \leq 0.001$, $p \leq 0.01$, and $p \leq 0.05$, respectively (Tukey's HSD test); ns, not significant.

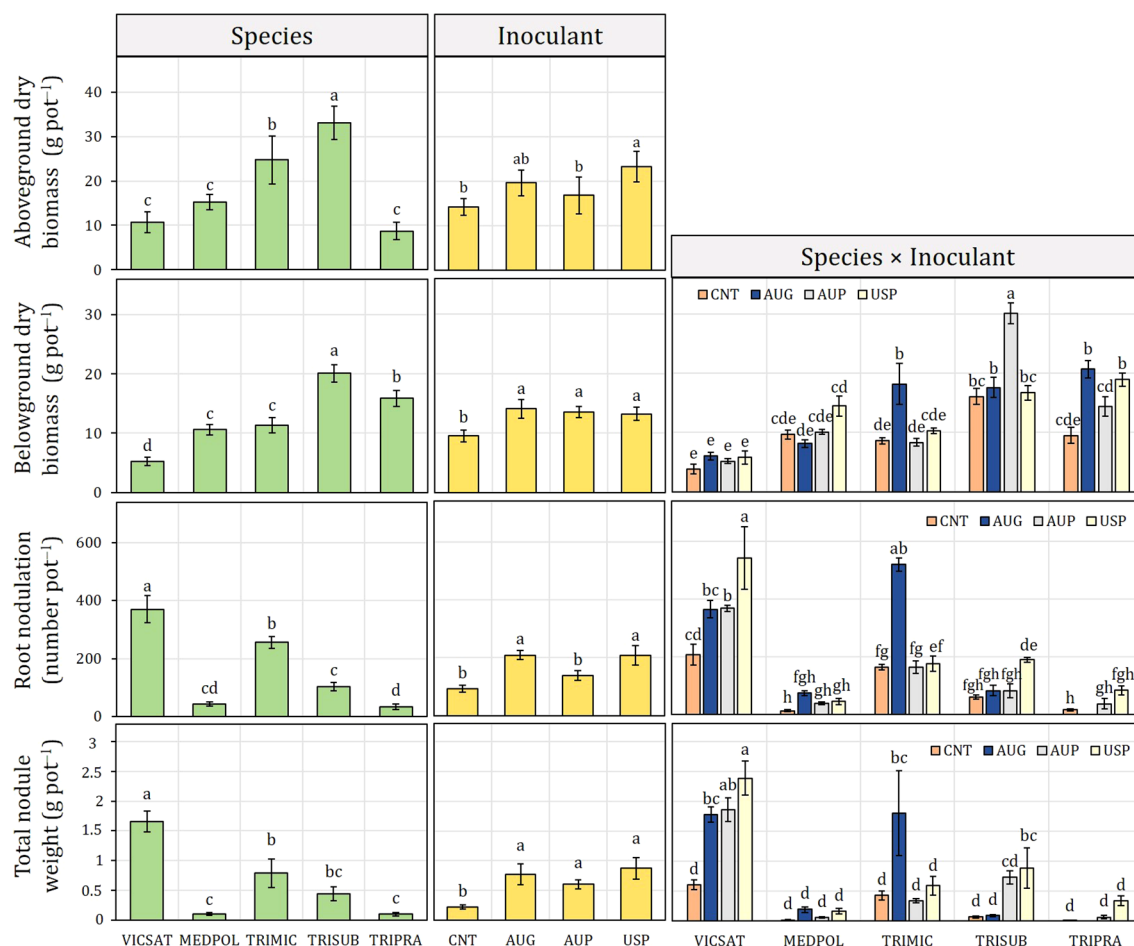


FIGURE 3

Inoculant formulation effect (non-inoculated control, CNT; Australian granular, AUG; Australian peat, AUP; American peat from Visjon Exceed®, USP) on aboveground and belowground dry biomass, root nodulation, and total nodule fresh weight of *Vicia sativa* (VICSAT), *Medicago polymorpha* (MEDPOL), *Trifolium michelianum* (TRIMIC), *Trifolium subterraneum* (TRISUB) and *Trifolium pratense* (TRIPRA) from Experiment B. Bars are standard error. Different letters indicate statistical significance at $p \leq 0.05$ (Tukey's HSD test).

(14.5 g pot⁻¹), for *T. michelianum* and *T. pratense* by AUG (18.2 and 20.6 g pot⁻¹, respectively), and for *T. subterraneum* by AUP (30.1 g pot⁻¹). All inoculants increased the belowground biomass versus the control, with no significant differences among inoculant types. Across the average of inoculants, *T. subterraneum* had the highest belowground biomass (20 g pot⁻¹), followed by *T. pratense* (15.9 g pot⁻¹), and *V. sativa* the lowest (5.1 g pot⁻¹).

Both root nodulation and total nodule weight were highly affected by the “species × inoculant” interaction (Table 6). USP induced the highest values in *V. sativa* (543 number pot⁻¹ and 2.4 g pot⁻¹), *T. subterraneum* (190 number pot⁻¹ and 0.3 g pot⁻¹) and *T. pratense* (84 number pot⁻¹ and 0.1 g pot⁻¹), while AUG in *M. polymorpha* (75 number pot⁻¹ and 0.2 g pot⁻¹) and *T. michelianum* (520 number pot⁻¹ and 1.8 g pot⁻¹) (Figure 3). Interestingly, AUG inhibited root nodulation on *T. pratense*. *V. sativa* had the highest root nodulation and total nodule weight, followed by *T.*

michelianum. Regardless of legume species, all inoculant formulations increased both parameters versus the control.

3.2.2 Relative N₂ fixation

The “species × inoculant” interaction was significant for ANY ($p \leq 0.01$), BNY ($p \leq 0.001$), and relative N₂ fixation ($p \leq 0.001$) (Table 6). Legume species had the greatest influence on variance for ANY and BNY, while N₂ fixation was more affected by inoculant formulation.

ANY and BNY showed the same trend, respectively, observed for aboveground and belowground biomass (Figure 4). Concerning ANY, the highest value was found in *T. subterraneum* (73.9 mg N pot⁻¹) and *T. michelianum* (47.9 mg N pot⁻¹) for legume species, and in USP (48.3 mg N pot⁻¹, +58.7% than CT) and AUG (43.3 mg N pot⁻¹, +42.4% than CT) for inoculant formulations. In particular, USP induced the highest ANY for *T. subterraneum* (96.8 mg N

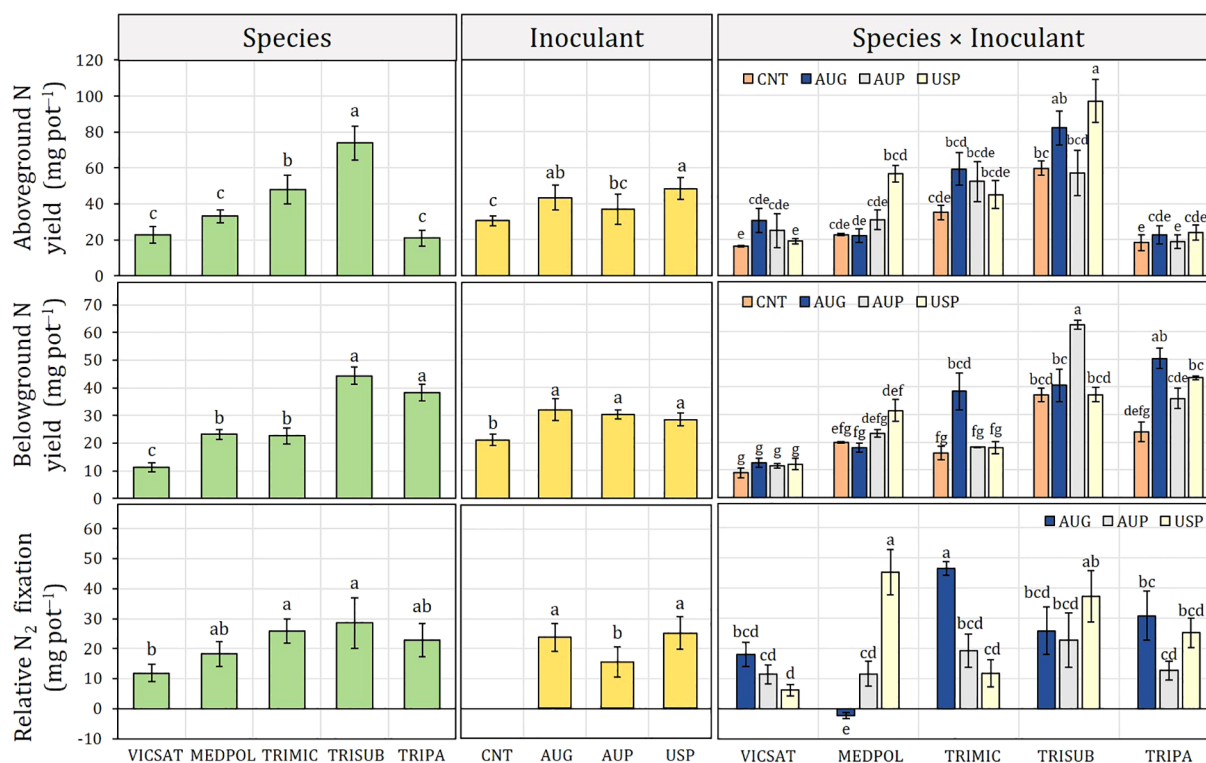


FIGURE 4

Inoculant formulation effect (non-inoculated control, CNT; Australian granular, AUG; Australian peat, AUP; American peat from Visjon Exceed®, USP) on aboveground N yield, belowground N yield and N₂ fixation of *Vicia sativa* (VICSAT), *Medicago polymorpha* (MEDPOL), *Trifolium michelianum* (TRIMIC), *Trifolium subterraneum* (TRISUB), and *Trifolium pratense* (TRIPA) from Experiment B. Bars are standard error. Different letters indicate statistical significance at $p \leq 0.05$ (Tukey's HSD test).

pot⁻¹), *M. polymorpha* (56.6 mg N pot⁻¹), and *T. pratense* (23.9 mg N pot⁻¹), whereas AUG was the best inoculant for *V. sativa* (30.7 mg N pot⁻¹) and *T. michelianum* (59.2 mg N pot⁻¹). Concerning BNY, *T. subterraneum* and *T. pratense* showed the greatest values (44.2 and 38.2 mg N pot⁻¹, respectively), while *V. sativa* confirmed as the poorest legume species (11.1 mg N pot⁻¹). Across the average of species, no significant differences were found between inoculant formulations, as observed for belowground biomass, all of which have increased BNY compared to CNT. In accordance with the belowground biomass trend, AUG determined the highest BNY in *T. michelianum* (38.4 mg N pot⁻¹) and *T. pratense* (50.3 mg N pot⁻¹), USP in *M. polymorpha* (31.4 mg N pot⁻¹), and AUP in *T. subterraneum* (62.4 mg N pot⁻¹), whereas no significant differences were observed in *V. sativa*.

Across the average of inoculant formulations, *T. subterraneum* and *T. michelianum* showed the highest relative N₂ fixation rates (28.6 and 25.7 mg N pot⁻¹, respectively) and *V. sativa* the lowest one (11.8 mg N pot⁻¹) (Figure 4). On the average of legume species, the relative N₂ fixation of AUG and USP inoculants was similar and higher than AUP. From the “species × inoculant” interaction emerged that AUG in *V. sativa*, *T. michelianum*, and *T. pratense* fixed N₂ at levels greater than the other inoculants (18.0, 46.5, and 30.7 mg N pot⁻¹), but performed worse than CNT in *M. polymorpha* (−2.3 mg N pot⁻¹). At the same time, *M. polymorpha* and *T. subterraneum* inoculated with USP had significantly higher

relative fixed N₂ than the other inoculants, with fixation rates of 45.4 and 37.2 mg N pot⁻¹, respectively.

4 Discussion

Positive effects of rhizobia inoculants on forage legume growth and nodulation have been previously reported (Carlsson and Huss-Danell, 2003; Shockley et al., 2004), including common vetch (Albayrak et al., 2006) and annual medics (Materon, 1988). The enhancement of plant growth induced by rhizobial inoculation could be attributed to the direct fixed N provided by rhizobia strains, the increase in plant nutrient uptake, and the production of plant growth-promoting hormones such as indole-3-acetic acid (Yadav and Verma, 2014; Allito et al., 2021). Although background rhizobial populations are common in Mediterranean regions due to the long history of forage legume cultivation, their N-fixing effectiveness varies widely (Rejili et al., 2012). Two main strategies can be pursued to enhance forage legumes N-fixation: inoculating with commercial elite strains or inoculating with selected native rhizobia. This research focused on the former strategy, given that most of these strains, such as the Australians, were selected for high effectiveness, desiccation tolerance, and persistence in harsh conditions, which are in part exacerbated by climate change and can adversely impact legume-rhizobia associations.

In the present study, inoculation with elite species-specific rhizobia strains and different inoculant formulations significantly improved plant growth and nodulation of the forage legumes investigated only in Experiment B.

The native rhizobial populations likely present in the substrate may have been sufficient in Experiment A, as evidenced by nodulation in the control plants. However, assuming that native rhizobia are always effective is misguided. Some fields lack a history of specific legumes, and certain rhizobial strains can form nodules without efficiently fixing N, as indicated by nodules that lack pink or red coloration, signifying low leghemoglobin content. From a practical perspective, farmers often assume adequate nodulation without examining root nodules, risking undernodulation and reduced N-fixation. Insufficient inoculation, combined with challenges such as monoculture, limited crop rotation, soil acidification, residual herbicides, and unpredictable climates, can significantly reduce legume productivity and N-fixation (Yates et al., 2024).

Only root nodulation and total nodule weight were affected in Experiment A, in which the AUG and the American peat inoculant (USP) showed, respectively, the best and the worst promoting effect among the inoculants. The optimal performance of AUG may have a dual explanation. First, higher efficacy of granular inoculants compared to liquid or peat-based powder inoculation treatments has been demonstrated by Kyei-Boahen et al. (2002) for chickpea, Rice and Olsen (1992) for alfalfa, and Ocumpaugh (1991) for arrowleaf clover, especially under unfavourable conditions for rhizobia survival. Furthermore, the long transport distance from Australia and the United States could have compromised the quality of peat inoculants, whereas granules could have maintained stable cell numbers (1×10^9 CFU g⁻¹). Second, native rhizobia strains may exert better performances than non-native commercial inoculants. Batista et al. (2015), for example, indicated that red clovers inoculated with the native isolate of *R. leguminosarum* bv. *trifolii* (strain 317) in Uruguay grasslands produced more biomass than those inoculated with the commercial strain U204 thanks to the increased nodulation competitiveness of indigenous isolate 317 than U204. A similar finding was reported by Roughley et al. (1976) for subterranean clover across five sites in New South Wales (Australia). However, the symbiotic performance of naturalised soil rhizobia is not always constant and can be compromised if diverse rhizobia populations have naturalised in soils, as demonstrated by Drew et al. (2011) for several annual clover species of Mediterranean origin.

Rhizobial inoculation was ineffective in its nodulating and growth promotion effects of *M. polymorpha*, in agreement with Charman and Ballard (2004), who concluded that none of the 222 screened lines of burr medic formed effective symbioses with a wide range of soil rhizobia. The reasons for poor nodulation are numerous and can be attributed to the high specificity of burr medic rhizobial strains, the inoculation of inappropriate rhizobia strains, the presence of competing indigenous rhizobia in the commercial soil substrate, or the decline of viable rhizobia contained in the inoculant. About the former aspect, it is known that rhizobia strains differ in nodulation effectiveness. Hence,

rhizobiologists are continuing to search rhizobia strains for improved N fixation and subsequent forage quality (Yates et al., 2021). More specifically, for an efficient translation to the field of the inoculant-strain selection, it should be considered the overall symbiont genotype $\times$ host genotype $\times$ environment interaction (Bellabarba et al., 2023). In this regard, selecting legumes with inherent diversity in growth potential, biomass production, growth habits, and life cycle length offers a range of options to fit specific management goals, such as forage production, soil cover, or improved BNF.

In Experiment B, a greater influence of the “species $\times$ inoculant” interaction than Experiment A was observed, denoting a higher species- and inoculant-dependent effect. Overall, the trend was similar to Experiment A, but the effects were more pronounced, probably by virtue of the double dosage of inoculant application on the one hand, and the different phenological growth stage on the other hand. Doubling the rate of application of inoculants is often reported as a good strategy to improve the nodulation effectiveness (Jakhar et al., 2018; Jesus et al., 2018). This improvement is generally suggested under poor soils with no legume history and soil pH ≥ 5.5 or where host plants are stressed (Farquharson et al., 2022). Increasing the application rates of peat inoculants on seeds enhances, in turn, the likelihood of sufficient rhizobia survival until plant germination. Moreover, it is known that the effects of rhizobial inoculation are exacerbated at flowering. Our results are consistent with Lamptey et al. (2014), who found that inoculated soybean plants harvested at flowering recorded higher fresh and dry shoot matter versus plants harvested at the vegetative stage. Hossain and Solaiman (2004) reported a similar finding for mungbean varieties.

Overall, AUG and USP showed the best growth-promoting effects and N fixation rates in Experiment B, with species-specific results. According to Zdor and Pueppke (1990), peat formulation may help protect rhizobial strains from adverse environmental effects versus liquid carriers. Moreover, granular inoculants, which contain less moisture, could offer even greater protection. Therefore, although all inoculants under investigation were applied directly to the seeds, granular and peat inoculants could have increased the strain survival. The worse results provided by AUP compared to USP may be related to the use of native rhizobia strains.

Interestingly, AUG inhibited *T. pratense* root nodulation. We suppose this negative effect may be attributed to the not-appropriate choice of rhizobial strain. In this regard, Valverde et al. (2005) isolated and described from *T. pratense* a novel *Trifolium*-nodulating species (*Phyllobacterium trifolii* sp. nov.) that produces nodules on *Trifolium* spp. and *Lupinus* spp. Rodríguez-Navarro et al. (2022) selected and characterized *Trifolium*-nodulating rhizobia for pasture inoculation in Spain, indicating that several *Rhizobium* species can nodulate *Trifolium* spp. better than *R. leguminosarum* bv. *trifolii* and that some *Trifolium* species growth could be improved through appropriate rhizobial selection.

In both experiments, aboveground and belowground N yields were respectively consistent with aboveground and belowground dry weights, with significant differences between forage legume species. *T. subterraneum* showed the highest aboveground and belowground N yields among the investigated forage legumes.

Similar N shoot concentrations were reported by Ovalle et al. (2006). To the best of our knowledge, root N content of forage legumes has been poorly investigated. It was estimated to be 15–111 kg N ha⁻¹ for temperate species. Nnadi and Haque (1988) reported root N contents of 2.43% for *V. benghalensis*, 2.25% for *V. dasycarpa*, 2.08% for *M. truncatula*, 2.01% for *T. steudneri* cv. Shola, 1.23% for *M. scutellata*, 1.10% for *Lablab purpureus* cv. Rongai, and 0.87% for *L. purpureus* cv. Highworth, with positive but not significant interactions between N concentrations in the roots and aerial parts. Most studies estimate N₂ fixation only from aboveground plant parts, but according to Danso et al. (1988) up to 60% of the total fixed N of forage legumes may derive from roots. Indeed, the variation in N₂ fixation may vary based not only on the method adopted but also on the plant parts analyzed, dry matter yields, and C/N ratio (Carlsson and Huss-Danell, 2003). In general, N₂ fixation is positively correlated to dry weights and high C/N ratios (Carlsson and Huss-Danell, 2003; Zhang et al., 2022), which explains why aboveground and belowground N yields did not reflect the N₂ fixation in the present work. In addition, considering the significant contribution of belowground N, as demonstrated by the high root:shoot ratios here obtained (data not shown), we consider our estimation of the total N₂ fixation more realistic than the sole shoots N₂ fixation. Although we did not use sterile soil in the present experiments for BNF estimation, a natural soil with its native microbiota allows for evaluating the effectiveness of the additional inoculum in a more realistic context. Indeed, a sterile soil not only eliminates natural rhizobia but also other beneficial microorganisms, altering microbial interactions and making it difficult interpreting data in relation to real agricultural soil conditions.

5 Conclusions

From this preliminary study, we concluded that inoculation of selected forage legumes (*V. sativa*, *M. polymorpha*, *T. michelianum*, *T. subterraneum*, and *T. pratense*) with specific inoculants at double the recommended dose may be an efficient approach to enhance plant growth, nodulation, and N₂ fixation. Unfortunately, there is still a low availability of elite commercial strains across the Mediterranean area due to the lack of rhizobia inoculant companies in Europe. The implementation in the Mediterranean agriculture of elite rhizobia strains supported by high-quality research, such as from Australia and the United States, could be a partial solution, avoiding long transport distances that could compromise the quality of inoculants. The cost of increased inoculant use is minimal compared to the potential risk in yield and profit due to unknown nodulation (lack of field knowledge by the farmer), poor nodulation, and N deficiency. Given the ongoing decline in the fertility of Mediterranean soils, this strategy could enhance legume production under restrictive conditions while reducing the need for mineral N fertilizers for subsequent crops. Future research steps will focus on field trials to validate the optimal forage legume × inoculant combinations identified here, particularly in intercropping with cereals, as it represents a promising agroecological practice for Mediterranean cropping systems.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

AS: Data curation, Formal analysis, Investigation, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. AM: Data curation, Investigation, Methodology, Writing – review & editing. FC: Data curation, Investigation, Methodology, Writing – review & editing. TM: Data curation, Investigation, Methodology, Writing – review & editing. CT: Conceptualization, Investigation, Methodology, Writing – review & editing. RY: Conceptualization, Investigation, Methodology, Writing – review & editing. ST: Formal analysis, Investigation, Writing – review & editing. CC: Data curation, Investigation, Writing – review & editing. MO: Conceptualization, Funding acquisition, Investigation, Project administration, Resources, Writing – review & editing. MF: Writing – review & editing, Data curation, Formal analysis. AV: Data curation, Writing – review & editing. DS: Methodology, Project administration, Resources, Validation, Writing – review & editing, Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation. FG: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing.

Funding

The author(s) declare that financial support was received for the research and/or publication of this article. This work was supported in part by the Italian Ministry of Foreign Affairs and International Cooperation, grant number US23GR18 (RE-FARM project, CUP: J43C23000110001).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

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OPEN ACCESS

EDITED BY

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RECEIVED 30 November 2024

ACCEPTED 22 April 2025

PUBLISHED 23 May 2025

CITATION

Lelei DK, Sultan MS, Kuboja NM, Mwangi LN
and Sinclair F (2025) Enhancing maize (*Zea mays*)
productivity through integrated soil
fertility management: a participatory
approach in the degraded soils
of Kigoma, Tanzania.
Front. Agron. 7:1537292.
doi: 10.3389/fagro.2025.1537292

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Enhancing maize (*Zea mays*) productivity through integrated soil fertility management: a participatory approach in the degraded soils of Kigoma, Tanzania

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Maize is a staple cereal for countries in sub-Saharan Africa, characterized by a low average yield of less than 1 ton per hectare in many smallholder farms across these countries. The low maize yield is attributed to poor soil fertility, poor crop management practices, poor post-harvest handling techniques, and erratic rainfall. The objective of the study was to investigate the effects of selected integrated soil fertility management (ISFM) technologies on soil chemical properties and maize yields following the use of the InPaC-S (Portuguese for Integração Participativa de Conhecimentos sobre Indicadores de Qualidade do Solo or Participatory Knowledge Integration on Indicators of Soil Quality) methodological approach. This methodological approach was employed to mobilize farmers through workshops and field experiments using selected integrated soil fertility management options: use of organic manure, lime, and nitrogen, phosphorus, and potassium (NPK) fertilizer. The experiment was laid out in a randomized complete block design (RCBD) with three replications, including manure, lime, NPK, lime + manure, manure + NPK, lime + NPK, and control. The results revealed significant differences between the treatments ($p < 0.001$) and sites ($p < 0.001$) for all studied growth parameters. The use of lime + NPK significantly increased maize yields by 149% ($p < 0.001$) compared to the control and influenced electrical conductivity, cation exchange capacity (CEC), organic carbon, total nitrogen, total phosphorus, and exchangeable bases. In turn, the cost of maize production (USD/ha) varied between treatments, ranging from 419.8 to 630.9 USD in the control and lime + NPK, respectively. The major costs included inorganic fertilizers, weeding, and land preparation, with inorganic fertilizers contributing the most to the total production cost. The net revenue in USD/hectare for the treatments was significantly ($p < 0.001$) highest for lime + NPK (\$1,260.90) and lowest for the control (\$339.60). A sensitivity analysis was performed on the net income, and the results suggest that as fertilizer costs increase, there comes a point where their use is no longer economically viable. Consequently, different ISFM options, such as the combination of lime and manure, lime alone, and manure alone, become

relevant. This empirical evidence concludes that the use of other integrated soil fertility management options will translate to a long-term improvement in food security and better livelihoods among communities. Future research should focus on scaling up/out these ISFM practices to further improve soil health, increase crop yields, and promote better livelihoods in sub-Saharan Africa.

KEYWORDS

maize production, smallholder farming, InPaC-S methodology, soil health, economic viability

1 Introduction

Maize is a major staple cereal for sub-Saharan Africa (SSA), serving as a primary crop for millions of smallholder farms. Despite its importance, maize productivity in SSA, including Tanzania, remains remarkably low, often yielding less than one ton per hectare, far below the potential yield of 4.0–4.5 tons per hectare (Wickama, 2017). The yield gap is attributed to a range of constraints, including poor soil fertility, soil acidity, and loss of soil biodiversity. These constraints are further exacerbated by the limited adoption of improved agricultural practices and technologies (Mesele et al., 2025; Silva et al., 2023; Zingore, 2023; Muindi et al., 2016).

These soil-related challenges are acute in regions like Kigoma, where intensive continuous cultivation on small landholdings, typically ranging from 1 to 2 hectares, results in nutrient depletion and soil degradation over time (Yaseen et al., 2024). Additionally, the inability of farmers to invest in inorganic fertilizers further amplifies this problem, creating a vicious cycle of soil degradation and low productivity (Wato et al., 2024; Wickama, 2017). The major concern is soil acidity, one of the primary factors hindering maize production in Kigoma (Farooqi et al., 2024). Furthermore, the extensive use of acidifying fertilizers such as diammonium phosphate (DAP) without adequate soil amendments only exacerbates this acidity problem (Shanka, 2020). As a result, the degradation of soil, coupled with low input agricultural practices, leads to reduced crop yields, endangering food security for smallholder farmers who rely on maize as their main source of income and nutrition.

In order to address these challenges, this study sought to explore and promote sustainable integrated soil fertility management approaches to restore soil health and enhance maize productivity in the Kigoma region of Tanzania. Specifically, the study aimed to achieve the following objectives: (i) assess the effects of organic and inorganic fertilizers on the chemical properties of the degraded soils of Kigoma region; (ii) evaluate the impact of integrated soil fertility management (ISFM) practices on maize yields in smallholder farms in the Kigoma region; (iii) evaluate the economic outcomes, particularly the net revenue resulting from adoption of ISFM practices [use of manure, lime, nitrogen, phosphorus, and

potassium fertilizer (NPK) alone, or in combination] by smallholder farmers of Kigoma region; and (iv) identify effective participatory approaches to engage farmers in integrated soil fertility management research and facilitate the adoption of these practices.

Given the constraints mentioned, there is an urgent need to explore sustainable soil fertility management approaches that can restore soil health and improve maize productivity. One such promising approach is the integration of organic and inorganic fertilizers (Yaseen et al., 2024), a core principle of ISFM. ISFM highlights the efficient and combined use of organic and inorganic resources to address soil fertility issues while enhancing crop production and maintaining long-term soil productivity (Dunjana et al., 2023; Kalibata et al., 2024; Khan, 2024; Mng'ong'o and Ojija, 2024). For example, organic materials such as manure and crop residues, when used alongside inorganic fertilizers such as NPK, have been shown to improve soil organic carbon, enhance microbial activity, and restore soil biodiversity, which are all essential for sustainable agricultural practices (Dunjana et al., 2023; Wamalwa, 2024; Yeboah et al., 2024; Liang et al., 2021; Ayuke et al., 2011). However, despite the proven benefits of ISFM in improving soil fertility and increasing yields, its adoption in regions like Kigoma remains limited. This limitation can be attributed to several factors, including a lack of awareness among farmers about the potential benefits of organic inputs, limited access to quality fertilizers, and inadequate information dissemination strategies (Kiprotich et al., 2024; Yeboah et al., 2024; Pamuk et al., 2014; Mtambanengwe et al., 2012). Furthermore, the majority of the existing research on ISFM has been conducted in experimental settings, with limited farmer involvement in the research process. This resulted in limited practical applications and adoption of the intended technologies (Snapp, 2002; Gwandu et al., 2014). This gap in dissemination and technology adoption highlights the need for more participatory approaches to research that involves farmers in the identification, testing, and implementation of soil fertility management practices.

Moreover, participatory research approaches have been shown to be effective in bridging the gap between research and practical application, as they facilitate the co-learning of farmers and researchers. Studies have demonstrated that when farmers are actively involved in a research process, they are more likely to

adopt new technologies and practices (Kurira et al., 2019; Sanginga et al., 2001). An example of such an approach is the InPaC-S (Portuguese for Integração Participativa de Conhecimentos sobre Indicadores de Qualidade do Solo or Participatory Knowledge Integration on Indicators of Soil Quality) methodology, which fosters co-learning between farmers and agricultural scientists to co-develop ISFM options that are both scientifically sound and locally suitable (Barrios et al., 2012). This methodology allows for the identification of “best-bet” options for soil fertility management that are tailored to the specific conditions and needs of smallholder farmers in the Kigoma region.

The integration of the InPaC-S approach in this study aims to address the soil fertility constraints in the Kigoma region through participatory research, identifying and promoting ISFM practices that can enhance soil quality, improve maize yields, and boost the economic sustainability of smallholder farms. This participatory framework distinguishes this study from previous research which often lacked farmer involvement in the research process. Additionally, while the use of combined organic and inorganic inputs has shown promise in improving soil fertility and microbial health, leading to higher yields in other parts of SSA (Iqbal et al., 2021; Liang et al., 2021; Chen et al., 2024; Mahmood et al., 2017), there is still limited information on the specific impact of these practices on soil chemical properties, maize production, and net revenue in degraded soils typical of Kigoma.

Therefore, this study seeks to bridge these knowledge gaps by assessing the effects of integrated organic and inorganic fertilizers on soil chemical properties and ISFM practices on maize yields and net revenue in the degraded soils of Kigoma. By exploring the role of ISFM in restoring soil fertility and increasing maize productivity, the study will contribute to sustainable agricultural practices and provide actionable recommendations for farmers, policymakers, extension services, and agricultural researchers. Thus, the results will offer important insights into promoting more widespread adoption of ISFM through participatory approaches in regions facing similar challenges.

2 Materials and methods

2.1 Location of the study area

The study was conducted in the Kigoma District, located in the Kigoma region in the western part of Tanzania. The region is situated along the shores of Lake Tanganyika (Figure 1) between the latitudes 3.6° and 6.5° south and longitudes 29.5° and 30.5° east (The Planning Commission Dar es Salaam and Regional Commissioner's Office Kigoma, 2016).

The Kigoma District experiences a tropical climate characterized by a unimodal rainfall pattern from late October to

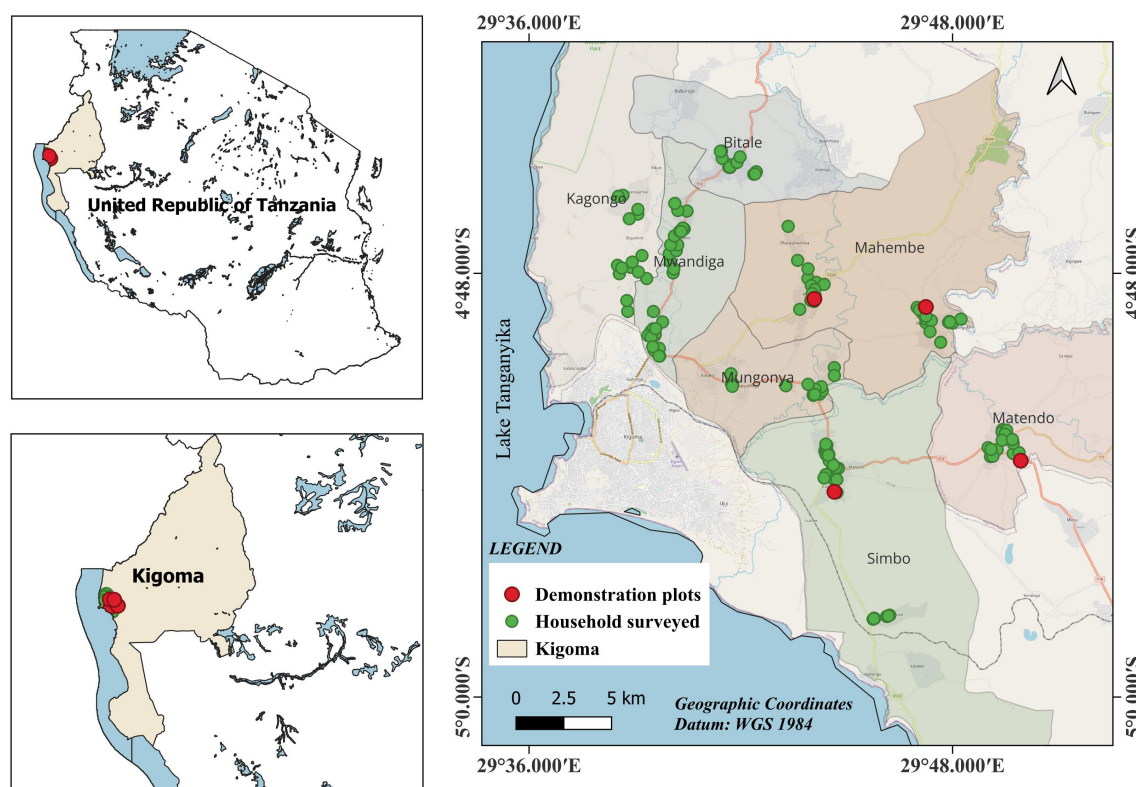


FIGURE 1
A map of Tanzania showing the study area.

May. The mean annual rainfall ranges from 600 mm to 1,500 mm, with an altitude ranging from 750–1,850 meters above sea level. Daily mean temperatures range between 25°C and 28°C, varying with altitude. During the cropping season, the average monthly temperature ranged from 21.7°C to 26.4°C, with the highest temperature recorded in November. The average monthly rainfall ranged from 99.7 mm to 350.8 mm, with the highest rainfall recorded in April and the lowest in January (Table 1).

Soils in the district vary by topography. Along the shores, they are deep, well-drained, and reddish brown fine sandy loams, but severely eroded. In low-lying areas, the soils are black and waterlogged, whereas higher relief areas contain black and brown alluvial soils. Well-drained dark reddish loams dominate other low-relief zones (The Planning Commission Dar es Salaam and Regional Commissioner's Office Kigoma, 2016; Mlingano Agricultural Research Institute, 2006).

2.2 Site selection

A baseline survey was conducted in 10 villages in the Kigoma District to assess the soil fertility status. Data collected from the field covered production constraints, knowledge of ISFM, land tenure system, crop productivity, fertilizer uses, soil types, and soil characteristics. The aim of the baseline survey was to assess and select sites with soil fertility constraints for the study. The Open Data Kit (ODK) tool was used for data collection (Ouma et al., 2019) using Android mobile devices. Based on low soil fertility among the 10 villages, four were selected to conduct demonstration trials, including, Kasuku, (latitude 4°54'11.358"S, longitude 29°44'39.156"E, and altitude 820m), Kidahwe, (latitude 4°53'18.42"S, longitude 29°44'39.156"E, and altitude 820m), Mahembe (latitude 4°48'43.5672"S, longitude 29°44'5.0352"E, and altitude 1012m), and Nkungwe (latitude 4°48'57.276"S, longitude 29°47'14.7048"E, and altitude 930m).

2.3 Selection of ISFM options

The selection of ISFM options was done in collaboration with farmers during workshop meetings that were undertaken

simultaneously with the baseline survey in the study area. Farmers selected ISFM technologies/options based on their soil conditions. In this study, different treatments were adopted, including manure, lime, NPK, lime + NPK, manure + NPK, and lime + manure, which represent various approaches to soil fertility management. However, according to the ISFM principles, true integration involves combining at least one of the organic inputs (manure) with inorganic fertilizer (NPK) or soil amendments (lime) to optimize nutrient availability and soil conditions. Therefore, treatments such as manure + NPK and lime + manure are examples of ISFM approaches, as they strategically integrate organic and inorganic amendments to enhance soil fertility, improve nutrient use efficiency, and support sustainable soil health. Therefore, the field experiments were conducted to validate the best-bet options among the soil management practices selected by the participants.

2.4 Field experiment

2.4.1 Soil sampling and analysis for field experiment

Soil sampling in the demonstration sites was conducted prior to planting and at harvest time. Five soil core samples were randomly collected at a depth of 0–20 cm (Anderson and Ingram, 1993; Santos et al., 2017) and thoroughly mixed to constitute a composite sample as described in Motsara and Roy (2008). A composite sample of approximately 1 kilogram from each site was air dried, ground, and allowed to pass through a 2.0 mm mesh. The soil samples were analyzed at the Tanzania Agricultural Research Institute's (TARI) Ukiriguru Center Soil Laboratory for particle size distribution, soil pH, cation exchange capacity (CEC), exchangeable bases (Ca, Mg, K, and Na), organic carbon (OC), total N, and extractable P.

Carbon and nitrogen were analyzed by thermal oxidation using a CN-analyzer [Flash 2000 NC analyzer (ThermoFischer Scientific, Cambridge, UK)]. Soil pH was measured with a soil:water ratio of 1:2.5 using a pH meter (Anderson and Ingram, 1993). CEC was determined using the ammonium acetate method. Furthermore, available P and exchangeable K, Ca, and Mg were extracted using the Mehlich 3 procedure (Mehlich, 1984) and determined by inductively coupled plasma (ICP) atomic emission spectroscopy (Isaac and Johnson, 1998).

TABLE 1 Average monthly rainfall, temperature, relative humidity, and average wind speed during the study period (2019/2020) in the Kigoma District.

Year	Month	Precipitation	Temperature	RH	WS
		(mm)	(°C)	(%)	(m/s)
2019	November	116.0	26.4	75.45	2.78
	December	158.5	22.8	85.98	2.29
2020	January	99.7	23.2	86.67	2.17
	February	257.6	24.0	89.81	2.31
	March	308.5	23.8	92.80	2.40
	April	350.8	21.7	93.02	2.51

2.4.2 Experimental design and treatments

The treatment selection was done following the InPaC-S methodological approach (Figure 2) of Barrios et al. (2012), where participants discussed the management options identified from the local indicators of soil quality (LISQ) integrated with technical indicators of soil quality (TISQ). LISQ are the visually observable and identifiable soil properties, features, and characteristics that are used for qualitative assessment of the soil quality status in a given area (Barrios et al., 2006, 2012; Doran, 2002; Doran and Zeiss, 2000). Once the LISQ and TISQ are integrated, they lead to the co-production of hybrid indicators, which are further categorized into permanent and modifiable soil properties. Modifiable constraints, such as low availability of water and nutrients, low or high pH, bulk density, and low organic matter, can be improved through targeted management practices. A distinction is made between the soil that can be modified in the short, medium, and long term based on the time required to achieve a significant reduction in the constraint identified. The methodological guide considers the time the constraints need to be modified in terms of years as follows: short term = less than 2 years; medium term = 2–6 years; and long term = more than 6 years. The distinction between the short, medium, and long term is necessary to facilitate the prioritization of management strategies that will be possible based on the farmer's capacity to use inputs. The ISFM options were then generated and captured in the

management options matrix tool (MOMT), which guided the tailoring of ISFM options to soil quality classes and farmers' capacity to use inputs. MOMT is the spreadsheet-based decision-making tool designed to apply a set of decision criteria to a variety of alternatives or strategic options (Barrios et al., 2012). The best-bet ISFM options were agreed during the national and sub-national workshops and were implemented in the demonstration plots.

The experiment was laid out in a randomized complete block design (RCBD) with three replications. Each block was comprised of seven plots, each 4.5 m x 4.5 m, with 1 m between plots and 2 m between blocks. Three seeds per hole were planted with a spacing of 0.75 m x 0.5 m, and, 21 days after emergence, thinning was conducted to retain two plants per hole to maturity. The test crop in the study was maize variety TH 501 bred at TARI Tumbi center, tolerant to maize streak virus, leaf blight, and rust, and suited for areas with an altitude of 0–1,400 meters above sea level (m.a.s.l) and rainfall of above 600 mm in medium to light, fertile, and well-drained soils.

The treatments comprised inorganic fertilizer, manure, and agricultural lime. The fertilizer used for basal application was N=13:P=24:K=12, while urea (46% N) was used as a top dressing. The manure was composted cattle manure with the following nutrient contents: 30% C; 1.5% N; 0.64 ppm of P; 0.8 cmol kg K; 1.4 Cmol kg calcium (Ca). The lime treatment consisted of high calcium limestone (CaCO₃) with 40% Ca (Table 2). Lime and

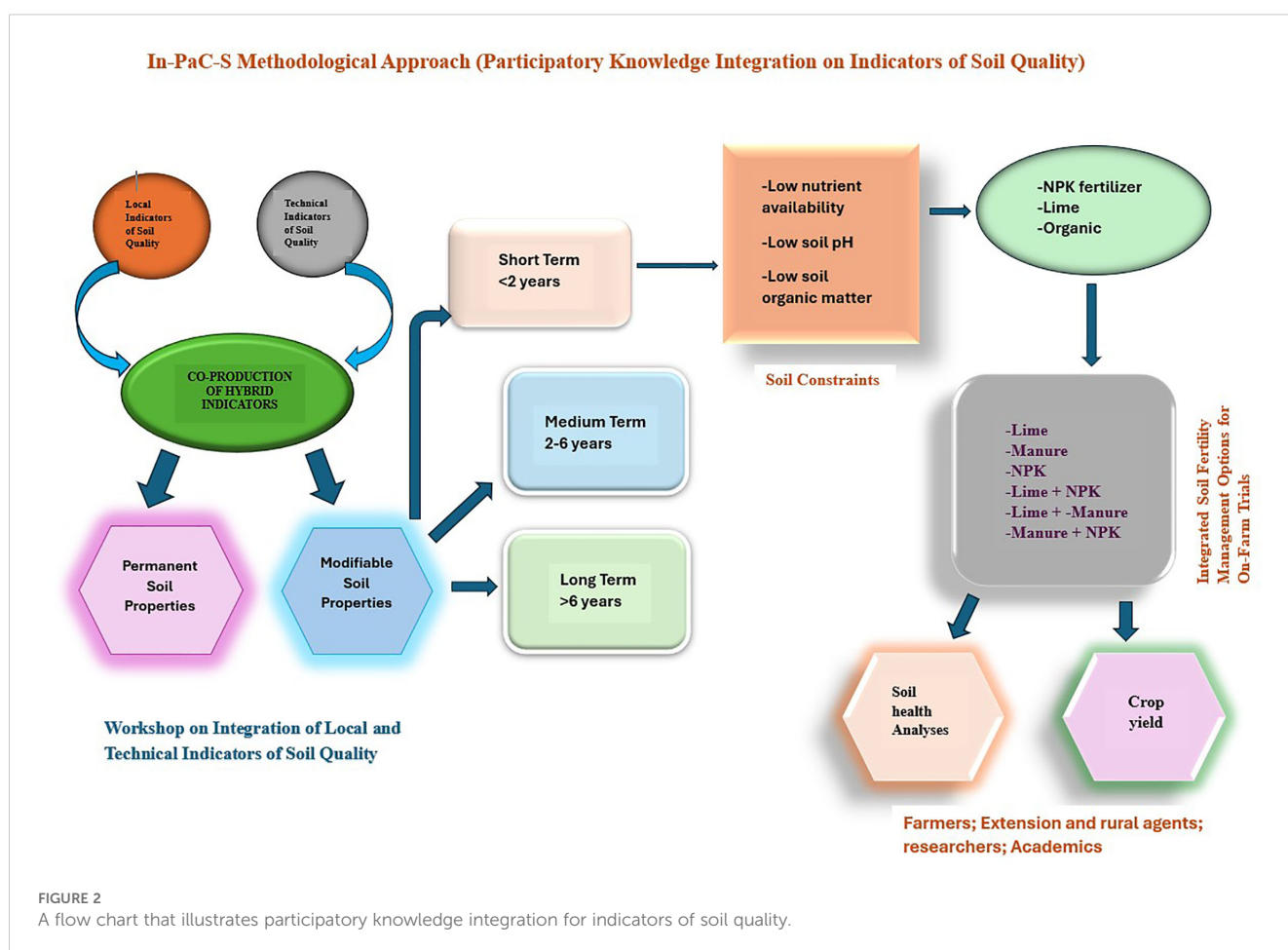


TABLE 2 Input treatments at Mahembe, Kidahwe, Nkungwe, and Kasuku sites during the 2019/2020 season.

Treatment	Application rates			
	Manure	Lime	NPK	Urea
Control	No input applied			
Manure	5 tons/ha	–	–	–
Lime (CaCO ₃)	–	3 tons/ha	–	–
Lime + manure	5 tons/ha	3 tons/ha	–	–
NPK + urea	–	–	104 Kg ha ⁻¹	101 Kg ha ⁻¹
NPK + urea + manure	5 tons/ha	–	104 Kg ha ⁻¹	101 Kg ha ⁻¹
Lime + NPK + urea	–	3 tons/ha	104 Kg ha ⁻¹	101 Kg ha ⁻¹

manure were spread and covered with the topsoil using a hand hoe 3 weeks prior to planting. The starter dose of NPK fertilizer was applied at a rate of 104 kg ha⁻¹, contributing 13.5 kg of N, 25 kg of P, and 12 kg of K ha⁻¹, at planting and placed at a 4 cm depth in each plot, and covered with soil before seed sowing. The second dose of urea was applied at a rate of 101 kg ha⁻¹, contributing 46.5 kg of N, and was done 3 weeks after the first weeding in plots that received NPK. During the growth and developmental stages of the maize plants, management practices, including thinning, weeding, fertilizer application, and disease control, were done accordingly (International Institute of Tropical Agriculture, 1982) (Table 2).

When the maize plants were mature and ready for harvest, plants were sampled from the central rows of each experimental plot at each site, with all edge rows excluded to prevent potential edge effects. Harvesting was done at the physiological maturity using standardized protocols. Plants were manually harvested, and key agronomic parameters, including maize grain yields, below and aboveground biomass, cob length (CL), plant height, thousand seed weight (TSW), and grain weight per plot, were recorded.

2.5 Data collection and analysis

During crop development, the following data were recorded: plant population plot⁻¹, plant height, and visual observations. During harvest, 20 maize plants were randomly collected from the central rows in each plot. Maize cobs were extracted, dried, and shelled, and the grains were dried to 12%–15% moisture content. The weight of grain harvested from each plot was determined, and the yield was expressed in tons per hectare (t ha⁻¹). Other yield parameters collected were plant height, CL, TSW, and above- and belowground biomass dry weight. Other socioeconomic data recorded were costs of production that included input and operation costs, and output prices. Tests for normality were performed using the Shapiro–Wilk test in R statistics, and where the data was not normally distributed, square root transformation of the data was done prior to data analysis. Analysis of variance (ANOVA) was performed on the collected variables using GenStat software version 18 (www.genstat.com; VSN International Ltd, Hemel Hempstead, UK). Additionally, Microsoft Excel was used

for a cost-benefit analysis, and a sensitivity analysis was conducted. These analyses aimed to establish the realized net returns and their stability across ISFM options.

3 Results

3.1 Initial soil properties

The analysis of the initial soil properties showed that the soil texture of the area was silty loam with moderate water holding capacity (Table 3). Generally, sandy soils have low moisture retention capacity, which is higher for clayey soils (Salter and Williams, 1965).

The soils from the study sites had CECs ranging from 1.74 to 5.20 cmolc (+) kg⁻¹ (Table 4). According to Landon (1991), CEC values less than 15 cmol kg⁻¹ are considered low. The low values of CEC in this study are directly related to the low organic matter content observed in the soil analysis. Soils with high CEC have a high surface area, which effectively comes into contact with water and soil nutrients. Soares and Alleoni (2008) and Kome et al. (2019) suggested that CEC is largely influenced by soil texture, clay content, and types of clay minerals.

The low values of exchangeable cations Ca (1.71–2.61 cmol kg⁻¹), Mg (0.22–0.58 cmol kg⁻¹), and K (0.03–0.05 cmol kg⁻¹) observed in this study can be attributed to the low CEC values recorded (Table 4). Similarly, according to Lambooy (1984), soils with low CEC will also have low OC (2.03%–2.54%), total nitrogen (TN) (0.11%–0.14%), and available P (10.00–12.30 ppm). The observed low soil nutrient values in all villages were attributed to very low organic carbon contents and low soil pH.

3.2 Effects of treatments on the soil chemical properties

The treatment of lime co-applied with fertilizer (lime + NPK) significantly increased soil pH. In contrast, there was a significant increase in EC, CEC, and exchangeable calcium in soils that received lime co-applied with manure (lime + manure), whilst manure applied alone significantly increased K.

TABLE 3 Initial soil properties (depth of 0–20 cm) prior planting in the four sites, Kigoma.

Soil properties	Site			
	Kasuku	Kidahwe	Mahembe	Nkungwe
pH (H ₂ O)	5.35	4.4	5.07	4.13
OC (%)	2.04	2.08	2.09	2.51
TN (%)	0.13	0.13	0.12	0.12
P (ppm)	11.65	12.35	10	12.05
EC (mS/Dm ³)	0.03	0.05	0.03	0.04
K (cmol/kg)	0.03	0.05	0.05	0.03
Ca (cmol/kg)	2.16	2.38	1.82	2.6
Mg (cmol/kg)	0.57	0.46	0.28	0.47
CEC (me/100g)	4.07	4.61	2.5	1.75
Sand (%)	77	58	74	37
Silt (%)	11	25	13	45
Clay (%)	12	15	16	19
Textural class	Silty loam	Silty loam	Silty loam	Loam

OC, Organic Carbon; TN, Total Nitrogen; P, Phosphorus; EC, Electrical Conductivity; K, Potassium; Ca, Calcium; Mg, Magnesium; CEC, Cation Exchange Capacity.

All treatments with lime, whether solely or in combination with NPK or manure, generally increased soil pH when compared to the control. Soil pH significantly differed ($p = 0.009$) among the treatments with lime + NPK, recording the highest pH of 6.51 compared to 4.48 in the control plots. The results also revealed that treatment had significant effects on EC ($p < 0.009$) and CEC ($p < 0.001$), with lime + manure recording the highest EC (0.12 me100g⁻¹) and CEC (9.18 me100g⁻¹) compared to 0.04 and 1.87 me100g⁻¹ in the control plots respectively, which translated to 300% and 490% increases, respectively (Table 5). Similarly, the lime + manure treatments had significant effects on exchangeable calcium, recording 5.38 cmol kg⁻¹ Ca as compared to 2.12 cmol kg⁻¹ in the control plots, which translated to a 326% difference. The sole manure treatment had significant ($p < 0.001$) effects on exchangeable K, recording the highest exchangeable K of 0.1 cmol kg⁻¹ compared with 0.03 cmol kg⁻¹ in the control plots and this translated to a 233% difference. The results also showed that available P was the lowest in the control compared to other treatments, however, there was no significant difference among the other treatments. NPK + manure recorded the highest phosphorus of 13.43ppm against 9.56ppm in the control, which translates to a 40.5% difference (Table 4; Figure 3).

The results also showed that available P was significantly ($p < 0.002$) lower in the control compared to other treatments. However, NPK + manure recorded the highest phosphorus content of 13.43ppm against 9.56ppm in the control, which translates to a 40.5% difference.

The results showed that total C was significantly ($p < 0.039$) lower in the control compared to the other treatments, but the other treatments did not record significant difference. However, it was noted that lime + manure recorded 28% higher total C compared to the control.

To further gain an insight into the results, a regression analysis was conducted with additional insights into the relationships between ISFM treatments and soil parameters. Both the ANOVA and regression analyses (Tables 4, 6) identified significant effects of ISFM treatments on soil pH and potassium. However, discrepancies between both analyses were observed for OC, calcium, and phosphorus. While ANOVA showed significant treatment effects on calcium and P, these effects were not evident in the regression analysis. In contrast, the regression analysis revealed a significant positive effect of the NPK+manure treatment on TN, a result that was not detected by ANOVA. This shows the role of combined organic and inorganic inputs in enhancing N retention.

3.3 Effects of the treatments on maize growth performance and yields

The analysis of variance results for treatments, sites, their interactions, and the mean effects of the treatments on growth performance and maize yields are presented in Table 7. The results showed a significant difference ($p < 0.01$, $p < 0.001$) for all growth parameters studied except for TSW. This implies that the treatments had a significant contribution to maize growth performance.

The lime + NPK treatment significantly influenced multiple maize growth parameters, including belowground biomass (BgB), grain weight per plot (GWP), plant height (PH), CL, aboveground biomass dry weight (AgB), and overall maize yields. Notably, the lime + NPK, NPK + manure, and NPK had significant effects on GWP, but no significant differences were recorded among the three treatments. However, lime + NPK (12.07 kgs) recorded the highest GWP difference compared to the control (4.82 kgs), with a 150% increase over the control. The increase in GWP compared to the

TABLE 4 Effects of lime, manure, and NPK fertilizers on the soil chemical properties.

Treatment	pH (H2O)	EC(Sm ⁻¹)	CEC (me 100g ⁻¹)	OC (%)	TN (%)	P (ppm)	K(cmol kg ⁻¹)	Mg(cmol kg ⁻¹)	Ca(cmol kg ⁻¹)
Control	4.48 ^a	0.04 ^a	1.87 ^a	1.85 ^a	0.12 ^a	9.56 ^a	0.03 ^a	0.32 ^a	2.12 ^a
Lime	6.26 ^{cd}	0.11 ^{bc}	5.36 ^{bc}	2.27 ^b	0.14 ^a	13.08 ^b	0.08 ^b	0.62 ^b	4.09 ^{cd}
Manure	5.51 ^b	0.07 ^{ab}	4.56 ^b	2.30 ^b	0.13 ^a	11.95 ^b	0.10 ^c	0.57 ^b	3.53 ^{bc}
NPK	5.35 ^b	0.08 ^{bc}	4.15 ^b	2.20 ^b	0.15 ^a	12.81 ^b	0.08 ^b	0.79 ^b	2.70 ^{ab}
Lime + Manure	5.60 ^b	0.12 ^c	9.18 ^d	2.37 ^b	0.43 ^a	12.19 ^b	0.08 ^b	0.76 ^b	5.38 ^e
NPK + Manure	5.84 ^{bc}	0.07 ^{ab}	4.74 ^b	2.26 ^b	0.43 ^a	13.43 ^b	0.08 ^b	0.75 ^b	3.69 ^{bc}
Lime + NPK	6.51 ^d	0.09 ^{bc}	6.70 ^c	2.35 ^b	0.15 ^a	12.63 ^b	0.07 ^b	0.72 ^b	4.88 ^{de}
CV	3.9	19.6	12.9	5.8	64.3	5.2	1.8	10.6	10.6
LSD	0.55	0.04	1.83	0.31	0.37	1.57	0.02	0.33	0.96
SE	0.18	0.01	0.62	0.11	0.12	0.53	0.01	0.11	0.32
<i>p</i> -value	0.001	0.009	0.001	0.039	0.298	0.002	0.001	0.093	0.001

EC, Electrical Conductivity; CEC, Cation Exchange Capacity; OC, Organic Carbon; TN, Total Nitrogen; P, Phosphorus; K, Potassium; Mg, Magnesium; Ca, Calcium.

p-values marked in bold are significant: **p*<0.05; ***p*<0.01; ****p*<0.001.

Mean followed by the same case lowercase letters are not significantly different at *p*<0.05.

TABLE 5 Treatments effects on maize net revenue.

Site	Net revenue (\$)
Nkungwe	237.60 ^a
Kasuku	893.40 ^b
Kidahwe	1,023.50 ^{bc}
Mahembe	1,147.70 ^c
Treatment	
Control	339.60 ^a
Lime	786.10 ^{bc}
NPK	970.30 ^{cd}
Manure	641.50 ^b
Lime + Manure	769.00 ^{bc}
NPK + manure	1,011.60 ^d
Lime + NPK	1,260.90 ^e
p-value	
Treatment	<0.001
Site	<0.001
Treat × Site	<0.001

Values in bold are significant: $p < 0.001^{***}$.

Mean followed by the same case lowercase letters are not significantly different at $p < 0.05$.

control in the different sites was as follows: Kasuku (141%), Kidahwe (103%), Mahembe (102%), and Nkungwe (736%). Similarly, both lime + NPK and NPK + manure recorded significantly higher TSW, but no significant differences were found between the two treatments. Lime + NPK recorded a 54% higher TSW compared to the control. Overall, the treatments and sites had a significant effect on all growth parameters except for TSW, indicating that the applied treatments contributed significantly to enhancing maize growth.

Significant variations in the maize yields and other crop parameters were observed across the four sites. Kidahwe recorded significantly higher BgB and AgB compared to the other sites, while Kasuku recorded significantly higher PH and CL. In contrast, Nkungwe recorded significantly lower yields, whereas the other three sites recorded higher yields, but there was no significant difference among them. However, Mahembe recorded the highest maize yield of 5.2 t ha^{-1} , with grain weight m^{-2} and grain weight plot^{-1} following a similar trend to that of maize yield.

Maize yield showed significant differences ($p < 0.001$) among the treatments, with lime + NPK recording the highest yield of 5.9 t ha^{-1} compared to 2.4 t ha^{-1} in the control, reflecting a 149% increase compared to the control. Lime + NPK consistently outperformed all the other treatments across all sites, recording the highest percentage increase in all parameters. Yield increases over the control across the different sites were as follows: Kasuku (141%), Kidahwe (103%), Mahembe (99%), and Nkungwe (736%). Similarly, the results also revealed that lime + NPK recorded the highest AgB of 4.3 kg compared to 2.1 kg in the control, which represented a 106% increase overall. Site-specific increases compared to the control were as follows: Kasuku

(239%), Kidahwe (74%), Mahembe (140%), and Nkungwe (46%). In contrast, there was a significant difference in PH in lime + NPK with 2.65 m compared to 1.66 m in the control plots, resulting in a height increase of 59.21% overall above the control. The different sites recorded increases compared to the control as follows: Kasuku (34%), Kidahwe (84%), Mahembe (55%), and Nkungwe (74%). Furthermore, BgB was significantly higher in lime + NPK, with a 178% increase compared to the control ($p < 0.002$). The site-specific increases in BgB compared to the control were as follows: Kasuku (646%), Kidahwe (74%), Mahembe (433%), and Nkungwe (62%). Finally, lime + NPK recorded the longest CL compared to the other treatments, which resulted in a 47.6% overall increase in cob length over the control. Site-specific increases in cob length compared to the control were as follows: Kasuku (40%), Kidahwe (41%), Mahembe (39%), and Nkungwe (79%) (Figure 4).

To further explore the relationships between soil and plant growth parameters across different integrated soil fertility management treatments, a correlation analysis was conducted. The results revealed several significant associations that show the factors influencing crop parameters (Figure 5). The correlations observed were consistent with the ANOVA results (Table 6), reinforcing the observed trends and interactions.

3.4 Cost-benefit analysis

Table 8 presents the net revenues that were calculated based on the maize yield from each treatment on a per-hectare basis. The cost of maize production ranged from USD 419.8 in the control to USD 886.70 for the NPK + manure plots. In the trial, the major costs included inorganic fertilizers, weeding, and land preparation. Inorganic fertilizer contributed the highest cost, ranging from 29.5% to 32.5% of the total production cost for plots that received inorganic fertilizers. Similarly, weeding and land preparation costs were high and cut across all the treatments, with weeding costs ranging from 20.0% to 32.7% of the total treatment cost in the inorganic fertilizer + manure and control, respectively, while land preparation costs ranged from 10% to 16.3% of the total treatment cost. Other costs included seeds, planting, pesticides, harvesting, shelling, packaging, and transport, which were generally lower. During the trial, an outbreak of fall armyworms was observed, and pesticide sprays were applied to eradicate them.

The market price of maize per 100 kg bag at the time of harvest was 32 USD (equivalent to TZS 77,965). This was used to determine the net revenue generated from maize production. The net revenue generated was significantly highest for Mahembe at USD 1,147.70 per hectare, while it was lowest for Nkungwe at USD 237.60. Similarly, across treatments, it was significantly highest for lime + NPK (USD 1,260.90) and lowest for control (USD 339.60), all on a per-hectare basis (Table 5).

Figure 6 presents the revenue generated when manure is purchased or not. Removing the cost of manure increases the revenue generated from the manure, lime + manure, and NPK + manure treatments by 64.4 USD ha^{-1} . The use of manure over the long-term by the smallholder farmers will improve soil fertility in their farms and hence their yields.

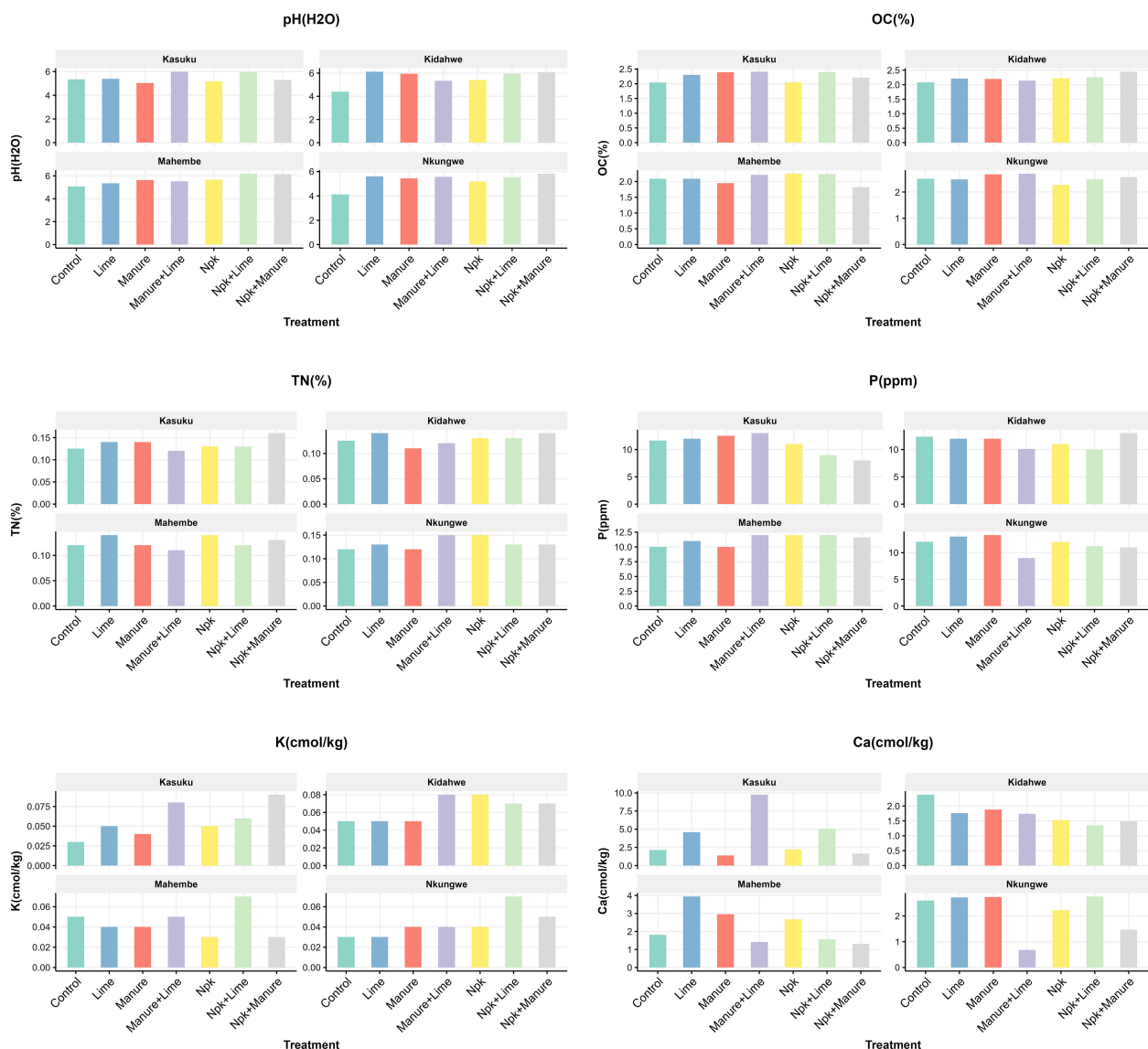


FIGURE 3
The bar graphs illustrate the impact of various treatments on soil fertility indicators.

3.5 Sensitivity analysis

Using actual maize production data from the study sites in the Kigoma District, we investigated the possibility of maize farmers maintaining positive net revenues despite increasing cost of production (for both organic and inorganic fertilizers). By examining the ISFM options considered in the study and incorporating yield effects and changes in production costs across options, a more realistic picture of a decrease in net revenues for each option was observed (Figure 7).

At a 1% increase in fertilizer prices, the use of lime + NPK resulted in the highest net return compared to other options such as lime + Manure, lime alone, and manure alone. However, as the rate of increase in fertilizer prices rose to 2% and above, the highest net revenue could be realized by farmers using lime in combination with manure, followed by those using lime and manure separately.

4 Discussion

4.1 Effects of treatment on soil pH and nutrient availability

The increase in pH could be attributed to neutralization of H^+ ions in the soil solution due to lime application (Khoi and Thom, 2015; Kisinyo et al., 2014; Chen et al., 2015; Kimiti, 2018; Mallarino, 2018; Corbett et al., 2021). The mechanism involves the dissociation of lime in the presence of water to Ca^{2+} , HCO_3^- , and OH^- ions, where H^+ ions are neutralized by HCO_3^- and OH^- , increasing soil pH. This shift towards neutral pH enhances the availability of base cations (Ca, Mg, and K), as documented by Qaswar et al. (2020); Mallarino (2018), and Tisdale et al. (2002). In addition, increased Ca^{2+} levels also result from the calcium present in the applied lime. Similarly, studies by Kisinyo et al. (2014); Chimdi et al. (2012);

TABLE 6 Regression analysis on the effects of lime, manure, and NPK fertilizers on the soil's chemical properties.

Variable	pH	OC (%)	TN (%)	P (ppm)	K (cmol kg ⁻¹)	Ca (cmol kg ⁻¹)	Mg (cmol kg ⁻¹)
Intercept	4.699***	2.16***	0.13***	11.19***	0.05***	3.58***	0.70***
Site							
Kidahwe	0.14	-0.03	-0.007	0.47	0.007	-2.10*	-0.44*
Mahembe	0.19	-0.16	-0.009	0.21	-0.01	-1.59	-0.30
Nkungwe	-0.13	0.27**	-0.022	0.63	-0.01		
Treatment							
Lime	0.88**	0.09	0.015	0.49	0.002	1.02	0.09
Manure	0.77**	0.12	0.00	0.44	0.002	0.00	-0.04
Manure+lime	0.86**	0.19	0.002	-0.49	0.02*	1.16	0.21
NPK	0.62*	0.02	0.015	-0.01	0.01	-0.08	-0.08
NPK+lime	1.18***	0.17	0.005	-0.96	0.03**	0.44	0.04
NPK+manure	1.10***	0.08	0.018*	-0.61	0.02*	-0.76	-0.19

*p<0.05; **p<0.01; ***p<0.001.

TABLE 7 Effects of the treatments on maize growth performance and yields.

Analysis of variance									
Variable	DF	BgB (kg)	GWP (kg)	GWM (kg)	PH (cm)	CL (cm)	TSW (kg)	AgB (kg)	Yield (t h ⁻¹)
Replication	2	0.04	0.65	0.01	76.8	0.63	0	0.84	0.15
Treatment	6	1.26***	72.94***	0.87***	12192.1***	47.58***	0.03***	8.26***	17.16***
Site	3	2.33***	127.27***	1.58***	3590.5***	72.29***	0.03***	18.27***	31.05***
Treat × Site	18	0.18***	6.99***	0.08***	1210.9***	1.04**	0	0.97***	1.65***
Error	54	0.06	1.58	0.02	138.9	0.44	0	0.2	0.37
Site									
Mahembe		7.1 ^a	10.6 ^b	1.2 ^b	215.7 ^a	15.4 ^{ab}	0.33 ^{ab}	21.9 ^a	5.2 ^b
Nkungwe		6.9 ^a	5.2 ^a	0.6 ^a	246.0 ^b	13.8 ^a	0.28 ^a	24.2 ^a	2.5 ^a
Kidahwe		14.1 ^b	10.0 ^b	1.1 ^b	237.2 ^{ab}	17.0 ^{bc}	0.35 ^b	42.4 ^b	4.9 ^b
Kasuku		9.1 ^a	9.2 ^b	1.0 ^a	246.0 ^b	18.1 ^c	0.35 ^b	26.0 ^a	4.4 ^b
Treatment									
Control		0.52 ^a	4.82 ^a	0.53 ^a	166.50 ^a	12.70 ^a	0.26 ^a	2.06 ^b	2.36 ^a
Lime		0.64 ^a	7.73 ^{bc}	0.85 ^{bc}	222.60 ^b	15.42 ^{bc}	0.30 ^b	1.76 ^a	3.77 ^{bc}
Lime + Manure		0.72 ^a	8.36 ^c	0.92 ^c	239.80 ^c	14.90 ^b	0.30 ^b	2.68 ^b	4.08 ^c
NPK		1.00 ^b	10.16 ^d	1.11 ^d	255.10 ^d	17.35 ^d	0.36 ^d	3.23 ^c	4.95 ^d
Manure		1.02 ^b	7.17 ^b	0.79 ^b	233.80 ^c	15.93 ^c	0.32 ^c	2.81 ^b	3.49 ^b
NPK + manure		1.17 ^b	10.83 ^d	1.17 ^d	237.30 ^c	17.42 ^d	0.38 ^e	3.27 ^c	5.27 ^d
Lime + NPK		1.43 ^c	12.07 ^d	1.32 ^c	265.10 ^e	18.75 ^e	0.40 ^e	4.25 ^d	5.88 ^e
CV (%)		4.3	14.4	14.3	5.1	4.1	3.4	6	1.7
LSD		0.39	2.06	0.22	19.3	1.09	0.4	0.73	0.99
SE		0.19	0.73	0.08	6.81	0.38	0.1	0.26	0.35

Where: BgB, belowground biomass dry weight; AgB, aboveground biomass dry weight; TSW, thousand seed weight; CL, cob length; PH, plant height; GWM, grain weight per meter square; GWP, grain weight per plot.

The following p-values are significant: **p<0.01; ***p<0.001.

Mean followed by the same case lowercase letters are not significantly different at p<0.05.

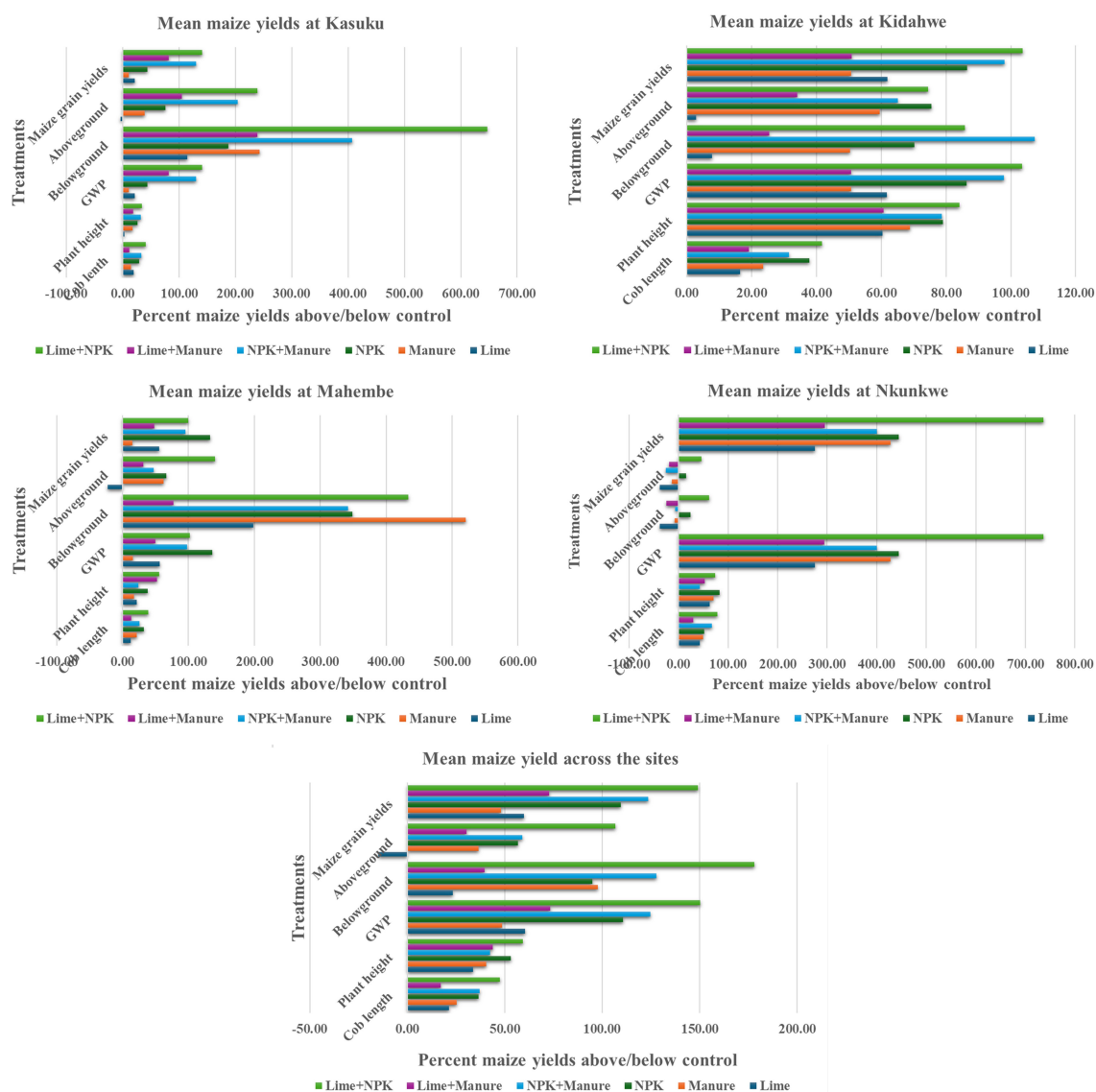


FIGURE 4
Effects of treatments on maize growth at different sites (Kasuku, Kidahwe, Mahembe, and Nkunkwe).

Verde et al. (2018), and Yaseen et al. (2024) confirm an increase in exchangeable Ca^{2+} following lime and fertilizer application.

Furthermore, manure application alone or in combination with lime enhances soil properties such as pH, Ca, Na, and microbial activities, as observed in studies by Qaswar et al. (2020); Otieno et al. (2018); Opala et al. (2018); Dhiman et al. (2019); Kisinyo et al. (2014); Chimdi et al. (2012), and Agbede et al. (2010). Manure and lime also improved available P levels, as increasing pH creates favorable conditions for P solubility (Yaseen et al., 2024; Verde et al., 2018; Kisinyo et al., 2014; Buni, 2014). The mechanism behind this is the release of exchangeable cations, potassium (K^+), calcium (Ca^{2+}), and magnesium (Mg^{2+}) during the decomposition of manure (Whalen et al., 2000). Additionally, Eghball et al. (2004) demonstrated that the buffering effect of manure plays a significant role in mitigating soil acidity. This effect is primarily facilitated through the decomposition process, where the presence of

bicarbonates and organic anions contributes to the neutralization of soil acidity and helps stabilize soil pH levels. Their findings align with the current understanding that manure can act as an effective buffer, promoting a more stable and less acidic soil environment.

These cumulative benefits of manure, however, do not occur instantly but take time to manifest. Its gradual effects on soil fertility are due to its impact on physical structure, increasing microbial diversity and nutrient mineralization (Zingore et al., 2008). These benefits result in increased maize yields and sustainable agricultural productivity (Fan et al., 2020).

Application of 6 t ha^{-1} of manure increased the CEC, resulting in increased base cations (Ca, Mg, and K) and available P, while reducing the toxicity level of Al and Mn (Ewulo, 2005). Similarly, Kheyroodin and Antoun (2011) documented improved soil fertility through nutrient addition, organic matter incorporation, and increased pH. However, recent studies by Tak et al. (2023) and

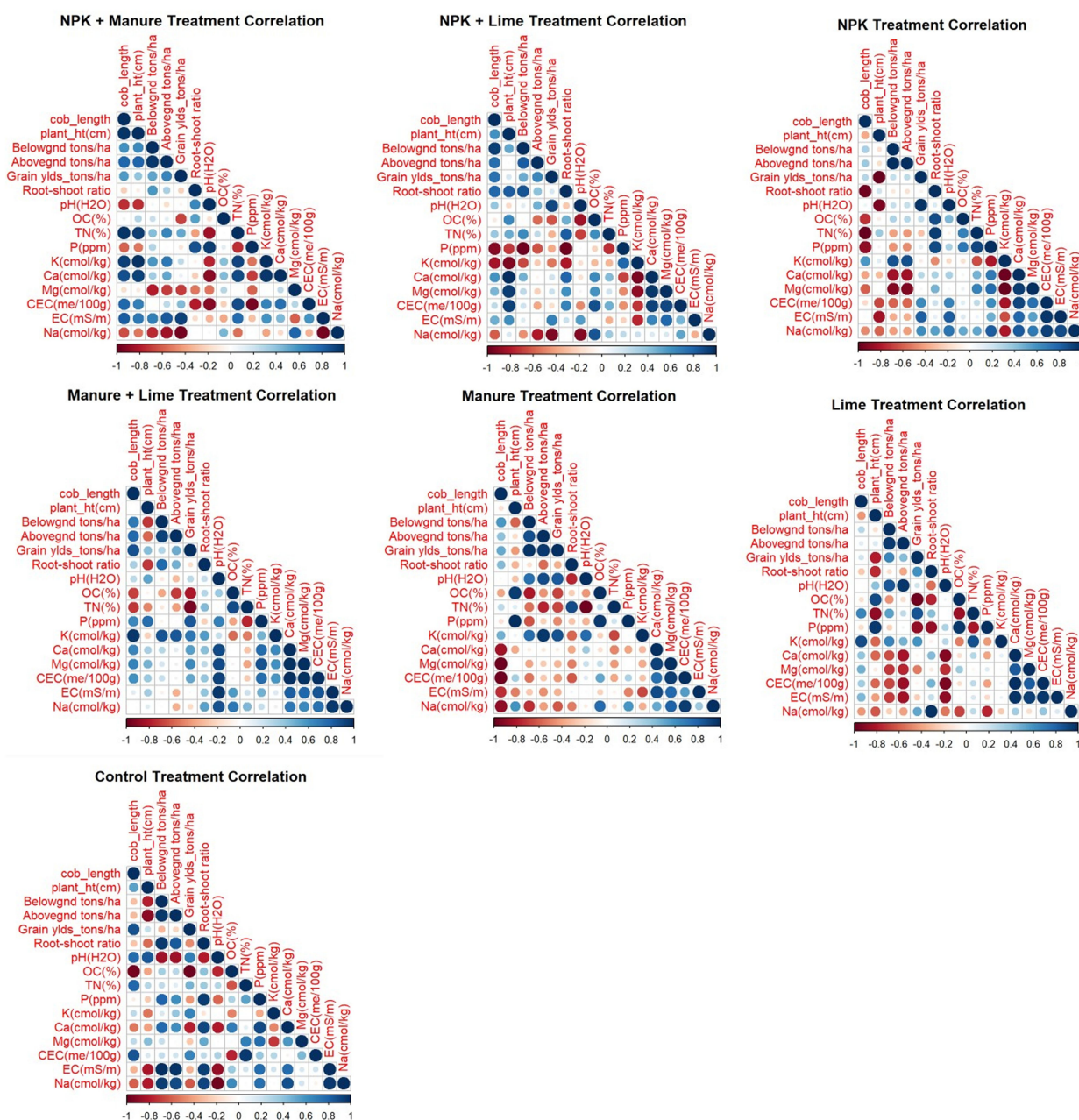


FIGURE 5

The correlations between different treatments and their effects on crop parameters and soil fertility indicators.

Cai et al. (2018) have further emphasized that the source and quality of manure play an important role in improving soil fertility and raising soil pH, confirming that manure's efficacy is highly dependent on its composition and treatment. Building on this, Kimiti (2018) and Azeez and van Averbek (2012) confirm that the quality of manure determines its efficiency in increasing soil pH. This could explain why manure (5.51) had a low capacity for soil pH increase in comparison to lime (6.26). The studies by Mugwe et al. (2009) and Whalen et al. (2000) corroborate that the application of organic manure led to an increase in soil pH, which they attributed to buffering from bicarbonates and organic

acids in cattle manure. A recent study by Shi et al. (2019) further corroborates this finding, showing that manure increased pH buffering capacity and the resistance of soil to acidification, resulting in stronger pH buffering. Furthermore, Kheyroodin and Antoun (2011); Adeniyi et al. (2011), and Agbede et al. (2010) documented that the use of manure and lime alone or in combination with fertilizers led to significant increases in Mg, Ca, and K, and resulted in reduced Mn toxicity in the soil. Recent research by Chen et al. (2021) and Verma et al. (2022) further validates these findings, showing that the combination of organic amendments with fertilizers improves nutrient cycling, reducing

TABLE 8 Cost (USD/ha) of maize production across the treatments.

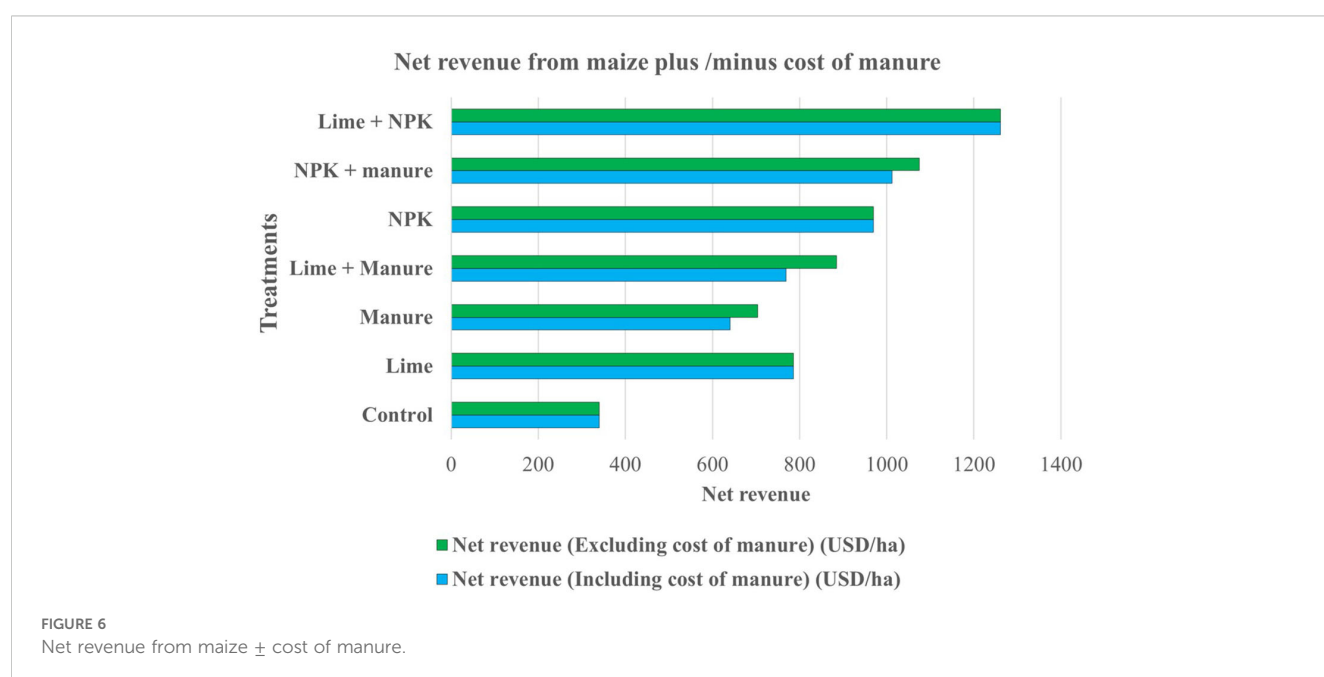
Cost	Control	Lime	Manure	Lime + manure	NPK	NPK + manure	Lime + NPK
Fertilizer	0 (0)	0 (0)	0 (0)	0 (0)	202.5 (32.5)	202.5 (29.5)	202.5 (32.1)
Lime	0 (0)	8.6 (2.0)	0 (0)	8.6 (1.7)	0 (0)	0 (0)	8.6 (1.4)
Manure	0 (0)	0 (0)	64.4 (13.3)	64.4 (13.1)	0 (0)	64.4 (9.4)	0 (0)
Land prep	68.6 (16.3)	68.6 (16.0)	68.6 (14.2)	68.6 (13.9)	68.6 (11.0)	68.6 (10.0)	68.6 (10.9)
Seed	42.9 (10.2)	42.9 (10.0)	42.9 (8.9)	42.9 (8.7)	42.9 (6.9)	42.9 (6.2)	42.9 (6.8)
Planting	25 (6.0)	25 (5.8)	25 (5.2)	25 (5.1)	25 (4.0)	25 (3.6)	25 (4.0)
Weeding	137.3 (32.7)	137.3 (32.0)	137.3 (28.4)	137.3 (27.9)	137.3 (22.1)	137.3 (20.0)	137.3 (21.8)
Pesticides	17.2 (4.1)	17.2 (4.0)	17.2 (3.6)	17.2 (3.5)	17.2 (2.8)	17.2 (2.5)	17.2 (2.7)
Harvesting	42.9 (10.2)	42.9 (10.0)	42.9 (8.9)	42.9 (8.7)	42.9 (6.9)	42.9 (6.2)	42.9 (6.8)
Shelling	32.2 (7.7)	32.2 (7.5)	32.2 (6.7)	32.2 (6.5)	32.2 (5.2)	32.2 (4.7)	32.2 (5.1)
Packaging	32.2 (7.7)	32.2 (7.5)	32.2 (6.7)	32.2 (6.5)	32.2 (5.2)	32.2 (4.7)	32.2 (5.1)
Transport	21.5 (5.1)	21.5 (5.0)	21.5 (4.4)	21.5 (4.4)	21.5 (3.5)	21.5 (3.1)	21.5 (3.4)
Productioncost	419.8 (100)	428.4 (100)	484.2 (100)	492.8 (100)	622.3 (100)	686.7 (100)	630.9 (100)

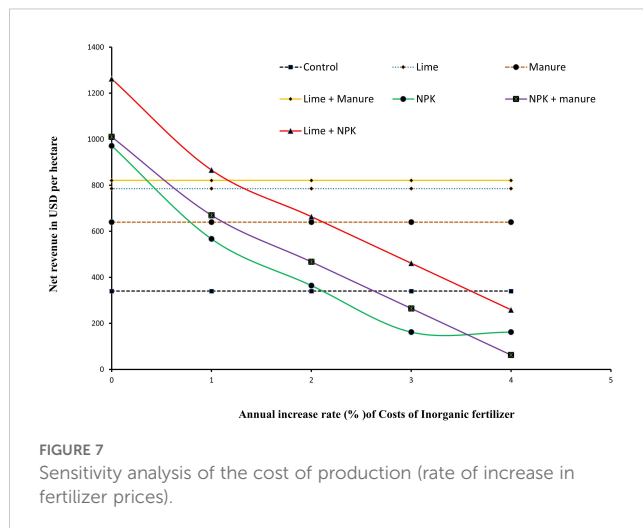
Values in brackets are percentages of the total cost of production for each treatment. All the plots that received NPK were top dressed using urea.

toxic elements accumulations and promoting soil health. The comparison of the ANOVA and regression analyses revealed similarities and differences in the evaluation of the effect of the ISFM treatments on soil parameters. Both methods identified significant treatment effects on soil pH, phosphorus, and potassium, aligning with previous studies showing the positive effects of lime and nutrient management on soil fertility (Kisinyo et al., 2014). However, discrepancies were observed for OC and TN. While ANOVA detected significant effects of the ISFM treatments on OC, the regression analysis did not, suggesting that a site-specific factor, such as soil texture, may have had a greater influence on OC

storage (Chivenge et al., 2007). The regression analysis identified a significant positive effect of the NPK + manure treatment on TN, which was not observed in the ANOVA results. This shows the advantage of regression analysis in elucidating treatment effects that account for site variability, which was not emphasized in ANOVA (Vanlauwe et al., 2010). Overall, the findings show the importance of using multiple statistical approaches to gain a comprehensive understanding of the effects of ISFM practices on soil properties.

In terms of organic carbon content, studies by Ndung'u et al. (2021) and Gram et al. (2020) documented that the application of manure + NPK significantly ($p < 0.05$) increased OC levels. This is





consistent with findings from [Sun et al. \(2015\)](#) and [Zhang et al. \(2024\)](#), which revealed that soil amended with livestock composts either alone or in combination with inorganic fertilizer had improved enzyme activity and bacterial diversity in soils. A recent study by [Das et al. \(2023\)](#) confirmed that livestock composts are not only crucial for improving soil health but also significantly enhance carbon sequestration. Finally, [Li et al. \(2017\)](#) showed that the combined application of manure and NPK fertilizers increased OC and TN and enhanced the bacterial communities that play important roles in the decomposition of complex organic matter and in transformations of soil carbon, nitrogen, and phosphorus. Recent work by [Zhang et al. \(2024\)](#) also confirmed the synergistic effect of combining organic and inorganic amendments, showing that such practices can further enhance microbial resilience and nutrient cycling in the soils.

4.2 Effects of the treatments on maize yield and crop parameters

Kigoma soils are generally acidic, requiring an application of lime to improve the soil's chemical properties and consequently, enhance maize yield. Lime plays an important role in ameliorating the effects of aluminum ions in the soil ([Muindi et al., 2015](#); [Kisinyo et al., 2014](#)). A study by [Haling et al. \(2010\)](#) has shown that soil acidity negatively affects root growth and soil nutrient sorption, which can lead to deficiencies in essential nutrients such as phosphorus and calcium. Lime increases soil pH, which facilitates aluminum hydrolysis, leading to precipitation as $Al(OH)_3$ and resulting in an increase in CEC, thus making exchangeable base cations (K and Ca) more available ([Tisdale et al., 2002](#)). Additionally, an increase in pH enhances P availability, an important nutrient for maize production. Studies by [Liang et al. \(2021\)](#); [Kimiti \(2018\)](#); [Sun et al. \(2015\)](#), and [Jabbar et al. \(2022\)](#) have documented similar findings that lime application, especially when combined with manure and NPK fertilizers, significantly improves nutrient availability and maize yield. Similarly, [Thakur et al. \(2020\)](#) and [Ayalew \(2010\)](#) also observed a maize yield increase following

the application of manure in combination with lime and mineral fertilizers.

Indeed, beyond the direct effects of lime, the integration of NPK fertilizers alongside lime forms an effective synergy that improves soil fertility and plant growth. The application of both lime and NPK has been shown to enhance a range of growth parameters, including BgB, aboveground AgB, PH, CL, and overall grain yield (Yield). Lime's effect on increasing soil pH not only facilitates the availability of nutrients but also enhances the efficacy of applied fertilizers by increasing the pH of the acidic soils ([Tisdale et al., 2002](#)). This synergy is evident in observed improvements in biomass production and the more robust root system, which are essential for nutrient uptake and overall plant growth ([Haling et al., 2010](#)). Moreover, the combination of lime and NPK fertilizers is an important strategy for addressing nutrient deficiencies in soils like those in Kigoma, which often limit the availability of nutrients such as P and Ca ([Liang et al., 2021](#)).

Site variability also played a substantial role in maize growth, with differences observed between locations such as Kidahwe, which had higher biomass production, and Kasuku, where plant height and cob length were superior. These differences highlight the importance of local soil conditions, such as soil texture and organic matter content, which can significantly influence the success of ISFM practices. A study by [Jabbar et al. \(2022\)](#) emphasized how localized characteristics, such as organic matter content and fertility, can impact fertilizer efficacy. Understanding site-treatment interactions can help tailor ISFM practices to specific regional conditions, thereby optimizing maize production in varying contexts.

The applications of manure, particularly when combined with lime and NPK, also contributed positively to maize growth. Manure improves soil structure, boosts microbial activity, and enhances nutrient cycling, thereby promoting sustained nutrient availability for maize plants. This aligns with findings by [Thakur et al. \(2020\)](#) and [Ayalew \(2010\)](#), who reported enhanced maize yield with the use of organic amendments. While manure alone improved maize growth, its combination with lime and NPK fertilizers produced even more significant results, emphasizing the synergistic effects of integrated nutrient management. Lime, by improving soil pH, likely unlocked the potential of organic amendments, facilitating better nutrient uptake.

The correlation analysis further compounds the importance of nutrient availability and soil fertility in driving maize growth, showing a strong relationship between plant height, biomass production, and nutrient levels in the soil. These findings emphasized the importance of soil amendments in improving soil health and enhancing maize productivity ([Liang et al., 2021](#)). Furthermore, understanding the role of decomposition of manure through microbial activities could offer further information on the mechanisms that lead to improved maize growth. A previous study by [Sun et al. \(2015\)](#) showed that microbial communities in organic-amended soils play a key role in nutrient cycling, which contributes to long-term improvements of soil fertility.

Therefore, the combined application of lime, NPK, and manure demonstrates an important strategy for improving maize growth in the acidic soils of the Kigoma region. Thus, by understanding these

complex interactions, it is possible to utilize ISFM practices to maximize yields and improve the overall soil health, resulting in improved food security.

4.3 Cost effectiveness of inputs used for maize production

The continuous use of acidifying fertilizer has hampered agricultural productivity growth among smallholder farmers in Tanzania. This is partially because of the negative attitude and lack of awareness by farmers towards fertilizer application. Moreover, poor farm management practices in Kigoma have contributed to soil and land degradation. Restoring soil health over time is important for farmers aiming to improve their yields and income.

The lower net revenue in the Nkungwe site may largely be attributed to poor crop yields due to degraded soils and waterlogging, both of which reduce soil fertility and hinder proper crop growth. In contrast, the highest revenue was generated from plots treated with NPK + lime. This outcome can be explained by the positive impact of lime, which helped neutralize soil pH, providing nutrients such as phosphorus and base cations to plants. Furthermore, lime similarly creates a more conducive environment for soil organisms, enhancing the overall soil structure and fertility. Additionally, the NPK fertilizer provided essential macronutrients, further boosting maize productivity. Although the use of inorganic fertilizers, such as NPK, delivers quick results in the short term, it is important to acknowledge the rising costs of these inputs. These increases are driven by factors such as the devaluation of the Tanzanian shilling against major currencies, higher transportation costs, and global fertilizer price inflation. As a result, while inorganic fertilizer may be cost-effective in the short term, the long-term sustainability of its use remains uncertain.

In contrast, organic amendments, such as manure, may take longer for soil fertility improvements to take effect, but they offer a more sustainable and cost-effective solution over time. The incorporation of organic matter into the soil can gradually restore fertility and improve soil health. A study by [Das et al. \(2023\)](#) supports this, emphasizing that while the benefits of organic fertilizers, such as manure, may take longer to manifest, they contribute significantly to long-term soil fertility enhancement. Smallholder farmers in Kigoma, who often lack the financial resources to purchase inorganic fertilizers, could greatly benefit from relying more on organic inputs. Moreover, manure, which is typically available from livestock, represents a vital resource for farmers to reduce their reliance on expensive inorganic fertilizers. To reduce the cost of production, smallholder farmers are encouraged to keep livestock that produce manure at a lower cost for use on their farms.

This finding suggests that as fertilizer costs increase, there may come a point where their use is no longer economically viable for smallholder farmers. Consequently, different ISFM options, such as lime + manure and lime and manure alone, may provide a more

economically sustainable solution. The findings of this study align with previous studies conducted by [Jjagwe et al. \(2020\)](#); [Islam et al. \(2019\)](#); [Singh et al. \(2019\)](#), and [Naeem et al. \(2006\)](#). Furthermore, these studies revealed that the use of organic amendments, e.g., manure and in combination with lime, had better soil performance than inorganic fertilizers, especially for soil fertility and sustainable crop productivity. Moreover, [Das et al. \(2023\)](#) emphasize the long-term benefits of manure in enhancing soil health and fertility. Therefore, adopting a combination of lime and manure could be a more cost-effective and environmentally sustainable approach for smallholder farmers in the region.

The sensitivity analysis presented in this study offers valuable insights into the future economic viability of different fertilizer strategies under varying price conditions. The analysis shows that if fertilizer prices increase by more than 2%, the use of lime combined with manure is the most cost-effective option. This suggests that the combination of organic inputs and lime can help farmers maintain higher net revenues, especially if organic inputs such as manure are incorporated into the farming system. The finding aligns with the broader literature on the cost-effectiveness of ISFM. Studies have shown that the ISFM approach not only enhances soil fertility but also improves the economic sustainability of farming systems in the long run ([Jjagwe et al., 2020](#); [Islam et al., 2019](#); [Singh et al., 2019](#)). As fertilizer costs continue to rise, smallholder farmers who adopt ISFM practices could be better positioned to maintain profitability. Inorganic fertilizers offer short-term benefits but their rising costs may make them less viable in the future. The adoption of organic inputs, particularly manure, alongside lime provides a more sustainable and cost-effective approach for smallholder farmers. Over time, organic amendments such as manure will improve soil fertility, leading to increased yields and reduced dependency on expensive fertilizers ([Luo et al., 2018](#)).

5 Conclusion

The study offers valuable insights on the influence of ISFM practices on soil chemical properties, maize growth performance, and economic returns in the region. The findings reinforce the important role of ISFM in addressing soil acidity and nutrient deficiencies. Specifically, the combination of lime and manure significantly improved soil pH, electrical conductivity, cation exchange capacity, and exchange calcium, which translated to enhanced maize growth and yield. The application of lime and NPK fertilizer resulted in the highest maize yields, demonstrating a 149% increase over the control treatments. The economic analysis revealed that while inorganic fertilizers remain costly, the use of manure and lime presents a more economically viable and sustainable alternative. This finding is of particular importance for smallholder farmers, as it offers pathways to improve productivity and profitability in the face of rising fertilizer costs. The sensitivity analysis further indicated the growing challenges posed by increasing fertilizer costs and supports the integration of organic inputs as a cost-effective and sustainable solution.

Given these findings, future research should focus on the long-term effects of ISFM practices, particularly the co-application of lime and manure on soil health and productivity under varying climatic conditions. Studies examining the optimal application rates of lime and manure and exploring synergies with other sustainable soil management practices would provide a deeper understanding, maximizing the benefit of ISFM. In addition, research into the socioeconomic barriers to widespread adoption of other practices among smallholder farmers, along with strategies to enhance their accessibility, would be valuable for scaling up ISFM adoption in developing regions. Ultimately, these efforts will contribute to the growing body of knowledge on sustainable agricultural practices and lay a foundation for promoting the use of ISFM approaches to improve soil fertility, crop productivity, and farmer profitability within resource-constrained smallholder farming systems in sub-Saharan Africa.

5.1 Study limitations

Our study was designed to fit a 1-year time frame allocated by the donor. The first phase involved conducting workshops to co-develop the research design with the stakeholders, while the second phase focused on implementing the trial at four sites. As such, the study was constrained to a single year, which restricted the possibility of collecting data across multiple seasons.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

MSS: Project administration, Data collection, Data curation, Data validation, Formal analysis, Investigation, Methodology, Writing-original draft, Writing-review and editing; DKL: Conceptualization, Project administration, Supervision, Data curation, Data validation, Formal analysis, Investigation,

Methodology, Data validation, Fund Acquisition, Visualization, Writing-original draft, Writing-review and editing; NMK: Formal analysis, Investigation, Validation, Visualization, Writing-review and editing; LNM: Formal analysis, Investigation, Methodology, Visualization, writing-review and editing; FS: Funds acquisition, Writing-review and editing.

Funding

The author(s) declare that financial support was received for the research and/or publication of this article. The work was supported and funded by World Agroforestry (ICRAF) and Forests Trees and Agroforestry (FTA).

Acknowledgments

The authors wish to acknowledge the World Agroforestry (ICRAF) and Forests Trees and Agroforestry (FTA) for the financial support and management through “Linking Knowledge to Action: Co-developing BEST-BET options for ISFM, increased profitability and poverty reduction in Agricultural Landscapes of Africa” project which enabled the implementation of this study in Kigoma, Tanzania; the Tanzania Agricultural Research Institute (TARI) for efficient implementation of the study; the Kigoma Regional and District authorities (LGA) for allowing and supporting the project; and the farmers for participating in and implementing the project.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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OPEN ACCESS

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RECEIVED 13 September 2024

ACCEPTED 24 April 2025

PUBLISHED 10 June 2025

CITATION

von Cossel M, Scordia D, Altieri M and
Gresta F (2025) Spotlight on agroecological
cropping practices to improve the resilience
of farming systems: a qualitative review of
meta-analytic studies.
Front. Agron. 7:1495846.
doi: 10.3389/fagro.2025.1495846

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Spotlight on agroecological cropping practices to improve the resilience of farming systems: a qualitative review of meta-analytic studies

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The capacity of agriculture to withstand or recover from increasing stresses (i.e., resilience) will be continuously challenged by extreme climate change events in the coming decades, altering the growing conditions for crop species. By prioritizing natural processes, agroecology seeks to foster climate change adaptation, boost resilience, and contribute to a low-emission agricultural system. Nineteen different agroecological practices using resilience-related terms and “meta-analysis”, within the subject areas ‘Agriculture and Biological Science’ and ‘Environmental Science’ were addressed, and 34 meta-analyses were reviewed to summarize the state-of-the-art agroecological adaptive strategies applied globally, and the current knowledge gaps on the role of agroecological practices in improving farming system resilience. Two main agroecological strategies stand out: i) crop diversification and ii) ecological soil management. The most frequent diversification practices included agroforestry, intercropping, cover cropping, crop rotation, mixed cropping, mixed farming, and the use of local varieties. Soil management practices included green manure, no-till farming, mulching, and the addition of organic matter. The analyzed studies highlight the complex interplay among soil, plant, climate, management, and socio-economic contexts within the selected agroecological practices. The results varied—positive, null, or negative—depending largely on site-specific factors. Developing and understanding more complex systems in a holistic approach, that integrates plants and animals across multiple trophic levels (feeding relationships, nutrient cycling, and aligning with the principles of a circular economy) is essential. More research is, therefore, needed to understand the interactions between crop diversity and soil management, their impacts on resilience, and how to translate research into practical strategies that farmers can implement effectively.

KEYWORDS

agroecological intensification, conservation agriculture, drought tolerance, intercropping, mixed cropping, soil management, sustainable intensification, traditional knowledge



system and critical climate goals like limiting temperature rise to 1.5° C (DG Agriculture, 2021; Farm to Fork; Biodiversity strategy as part of the EU Green Deal; CAP, 2023). A new partnership between the European Union and the Organization of African, Caribbean, and Pacific States champions agroecology's potential to safeguard biodiversity, nurture healthy ecosystems, and empower communities (European Commission, 2023).

To summarize the state of the art of agroecological practices to enhance agricultural adaptation to climate change employed worldwide, we aimed to identify current knowledge and knowledge gaps in the role of agroecological strategies (crop diversification and ecological soil management) in improving the resistance and resilience of farming systems. This overview intends to contribute to ongoing agroecological research by qualitatively synthesizing the results of meta-analytical studies on agroecological practices. While meta-analyses offer broader insights compared to individual studies, they face challenges such as heterogeneity of data, potential bias, multivariate effects, limited coverage, inclusion of low-quality studies, and the risk of oversimplified or misleading estimates when combining different causal factors (Eysenck, 1994). Individual studies, though informative, often provide site-specific results that may lack reproducibility due to variations in local factors like genetic material, equipment, soil conditions, and climate.

With the advancement of more rigorous meta-analytic methods, their application has expanded, including in ecology. Meta-analyses are now essential not only for synthesizing evidence but also for guiding research design (Borenstein et al., 2009). As the body of published research continues to grow, they play a crucial role in evaluating existing knowledge, identifying research gaps, and refining study methodologies by highlighting the most effective approaches from previous studies. Building on this, the present study aims to discuss existing quantitative syntheses and contribute to the ongoing debate on agroecological practices.

2 Agroecological practices selection

A literature review on 19 different agroecological practices (adapted from (Altieri et al., 2017) to enhance the resilience of agro-ecosystems was carried out on 11 December 2023 using the Scopus® database (Elsevier B.V.) (Supplementary Table 1). The asterisk (*) was used where necessary to find similar spellings of the respective agroecological practices. Primary literature was identified by the “Preferred Reporting Items for Systematic Reviews and Meta-Analyses” method (Page et al., 2021). A refinement using “resilience-related terms” was then carried out, and to further specify the document type, the search string was adapted by adding the term “meta-analysis”. Here, “conservation *agr*”, “minimum till*”, and “no *till*” refer to reduced tillage concepts without herbicide application. Although the use of synthetic plant protection products, such as herbicides and pesticides, is not explicitly prohibited within the framework of agroecology, this work focuses on practices that entirely avoid the use of synthetic products. The search was limited to the subject areas ‘Agriculture and Biological Science’ and ‘Environmental Science’.

In this review, the term metaanalysis was included since this type of study combines and statistically analyzes large amounts of data and can offer a clear overview of the impact of a specific treatment over control at a wider scale (Philibert et al., 2012). The total number of documents including meta-analytic studies of agroecological practices and resilience-related terms was 252. These documents were screened firstly for title and abstract, and 199 documents were removed due to various reasons such as (i) no meta-analysis, (ii) focus on other topics (sustainability assessment, modeling studies, etc.), and (iii) non-alignment (e.g., mineral fertilizers allowed, chemical herbicides and pesticides, etc.) with the agroecological farming concept.

The full text of remaining 59 documents were screened and new metaanalyses were identified from other sources such as the reference lists of the documents. In total, 34 meta-analyses were included in this review (Supplementary Table 2).

3 Results and discussion

3.1 Crop diversification

3.1.1 Agroforestry

Agroforestry is a crop diversification practice that integrates trees with field crops or pastures (Figure 1). Ngaba et al. (2024) thoroughly (n=125) investigated agroforestry effects on sustainable soil development at a global scale (Figure 2). Across environmental zones, major drivers contributing to global soil fertility were climatic conditions, agroforestry management, tree species selection, biodiversity, crop species selection, soil management, water management, farmer collaboration and training, socio-economic factors, policy support and markets (Figure 2). The meta-analysis of Scordia et al. (2023) on different agroforestry systems across Mediterranean countries (n=161) argued for a negative effect of trees on crop yield that could be ascribed to the competition for light. However, the % change of agroforestry as compared with monocropping was significantly different with tree type (i.e., from -75.8% in ash tree to +3.3% in walnut), with tree cover (from -33.5% with ≥ 200 trees ha⁻¹ to -8.2% with ≤ 99 trees ha⁻¹), and with associated crop species (i.e., from -80.8% in the faba bean to +4.5% and +13.1% in the barley and winter wheat). The potential benefits of agroforestry systems under anticipated extreme climate events in the Mediterranean region have been highlighted. While direct evidence of enhanced benefits during such events remains limited, it is hypothesized that the presence of trees may mitigate climatic extremes by reducing wind speed, lowering air temperature, and decreasing crop evapotranspiration (Kanzler et al., 2019; Markwitz et al., 2020). Additionally, in the absence of water stress, moderate shading provided by trees could improve the microclimate for associated field crops, potentially enhancing their resilience and productivity (Scordia et al., 2023).

A recent study by Rodenburg et al. (2022) explored the potential of integrating trees with rice production in Africa. They identified several tree species with broad adaptability and positive effects on rice yields, including *Sesbania rostrata*, *Aeschynomene afraspera*,



FIGURE 1

A silvopastoral agroforestry system experimental field (agroforestry in grasslands) set up in 2009 on the Swabian Alb in south-western Germany is investigating the potential of different woody crop systems to promote the resilience of a agroforestry system on a shallow soil (Rendzina). In this trial, short-rotation willow plantations left (a fast-growing biomass source) are compared with a mixture of local wild tree species right (photo courtesy of Moritz von Cossel).

Acacia auriculiformis, *Gliricidia sepium*, and *Gmelia arborea*. The study found that across all tree-rice systems, rice yields increased by an average of 38% compared to fields without trees. The average tree effect on rice yield (fertilized) was to increase yield by 261 kg ha⁻¹ equivalent to a +23% increase at low baseline rice yields (<1500 kg ha⁻¹). However, when the baseline yield was higher (>1500 kg ha⁻¹), the average effect of trees was to decrease rice yield by 519 kg ha⁻¹, equivalent to a decrease of 12%. Notably, some practices provided greater benefits. Biomass transfer and pre-rice green manuring in rice-trees system consistently improved yields. Hedgerow alley-cropping also showed promise, especially when fertilizers weren't used. In fertilized conditions, tree-crop competition negatively impacts yield in systems like hedgerow and intercrop, while non-competing systems (biomass transfer and pre-rice green manuring) show positive exceptions. This suggests that in high-yielding environments, trees may hinder rather than support crop productivity, posing risks to smallholder livelihoods.

Additionally, some tree integration methods like the short fallow practice showed rice yield reductions with fertilizer use. These findings highlight the importance of considering both the type of tree-rice integration and fertilizer use for optimal results. Rodenburg et al. (2022) call for further research to explore the broader environmental, social, and economic impacts of different tree-rice integration methods.

Several other scientific studies have examined the interactions between trees and crops grown in agroforestry systems. These studies shed light on key aspects:

- Firstly, the type of tree species and its root system can significantly influence crop yields. Research by Rivest et al. (2013) indicates that trees with deep tap roots, particularly those that fix nitrogen (N) like *Acacia* species, can benefit nearby crops during droughts through a process known as hydraulic lift. In contrast, trees with shallow root systems, like *Eucalyptus*, compete with crops for water, potentially reducing crop production. Interestingly, studies on scattered deciduous and evergreen oak trees showed no net change in pasture yields (Rivest et al., 2013).
- Secondly, the distance between crops and trees within an agroforestry system plays a critical role in determining crop yield. Meta-analyses by both Van Vooren et al. (2016) and Ivezić et al. (2021) highlight the importance of this spatial arrangement. In temperate alley-cropping and hedgerow systems, crop yields ranged from 70% over a distance of 1.64 times the tree height (when planted very close to trees) to 107% between 1.64 and 9.52 times the tree height (Van Vooren et al., 2016). Ivezić et al. (2021) modeled a 0.56% relative crop yield increase by each additional meter distance to the nearest tree.
- Thirdly, Ivezić et al. (2021) identified additional factors that can influence crop yield in agroforestry systems. Their research suggests that crop yield likely decreases with increasing tree density and tree age within alley-cropping systems. Furthermore, cereal crops generally outperform fodder crops when grown alongside trees in these systems.

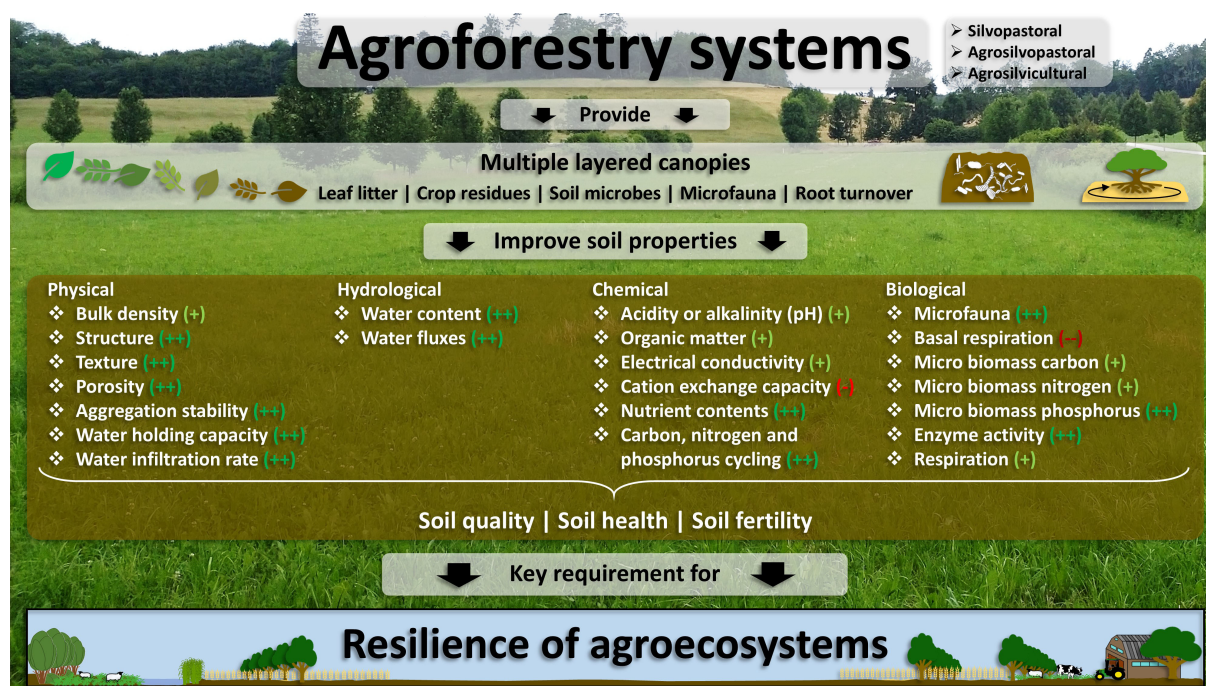


FIGURE 2

Agroforestry-mediated soil amelioration: a graphical representation. This diagram summarizes the hypothesized effects of various agroforestry techniques on soil characteristics. Directional arrows denote causal links, with symbols signifying the anticipated level of improvement across diverse climates: (–) no improvement across all climatic zones, (–) no improvement in specific climatic zone, (+) improvement in specific climatic zone, (++) consistent improvement in all climatic zones. Key soil variables include organic matter (OM), electrical conductivity (EC), and cation exchange capacity (CEC) (adapted from: Ngaba et al., 2024).

Interestingly, the relative response of crop yield appeared similar in both northern and southern European agroforestry settings (Ivezić et al., 2021).

- Finally, a recent study by Koutouleas et al. (2022) focused on the impact of shade on coffee production in agroforestry systems. Their meta-analysis disclosed significant variations in how different coffee cultivars respond to shade. Some coffee varieties showed no change in yield with shade, while others exhibited an inverted U-shaped response (highest yield at a specific shade level) or a continuous decrease with increasing shade. This research underlines the importance of considering the specific coffee cultivar when assessing its suitability for shade-grown coffee production within agroforestry systems. The authors also call for further research comparing coffee productivity across a wider range of low to moderate shade levels (10–40%) to potentially identify optimal shade levels for different coffee varieties.

3.1.2 Intercropping

Intercropping is a well-known crop diversification practice of growing two or more crops on the same field at the same time aiming at both overyielding effect (land use equivalent ratio > 1) and improved agrobiodiversity (Figure 3). A meta-analysis by Rodriguez et al. (2020) found that planting grain cereals alongside grain legumes boosts agricultural sustainability. This approach encourages plants to utilize more natural N sources, reducing the

need for external fertilizers. However, the success of intercropping depends on the specific mix of crops and how scientists measure N fixation. The study uncovered that intercropping significantly increased the total N uptake by the soil compared to sole legume crops (by an average of 25%). Interestingly, there wasn't a major difference in N uptake between intercropped and sole cereal crops. The real benefit came for the cereals themselves – intercropping significantly boosted their N uptake compared to monocrop (by an average of 61%). The study also explored how the proportion of cereals and legumes in the intercropping system affected N fixation. Interestingly, when compared to sole legume crops, intercropped legumes fixed slightly more N overall (an average increase of 14%). However, when considering the total amount of N fixed per unit area, intercropping reduced fixation by about 15%. This is because intercrops typically have a lower proportion of legumes compared to sole legume crops. To account for this difference, researchers adjusted the data to reflect the actual number of legumes planted in each system highlighting the importance of considering the amount of legumes planted.

Another meta-analysis examined 69 different systems where grasses, cereals, and legumes were grown together (grass-grain legume intercrops) (Bybee-Finley et al., 2016). It was found that intercropping led to more consistent yields compared to growing these plants separately (sole crops). The results showed a clear advantage for intercropping, with coefficients of variation of 0.25 for grass monocultures, 0.30 for legume monocultures, and just 0.19 for intercrops.

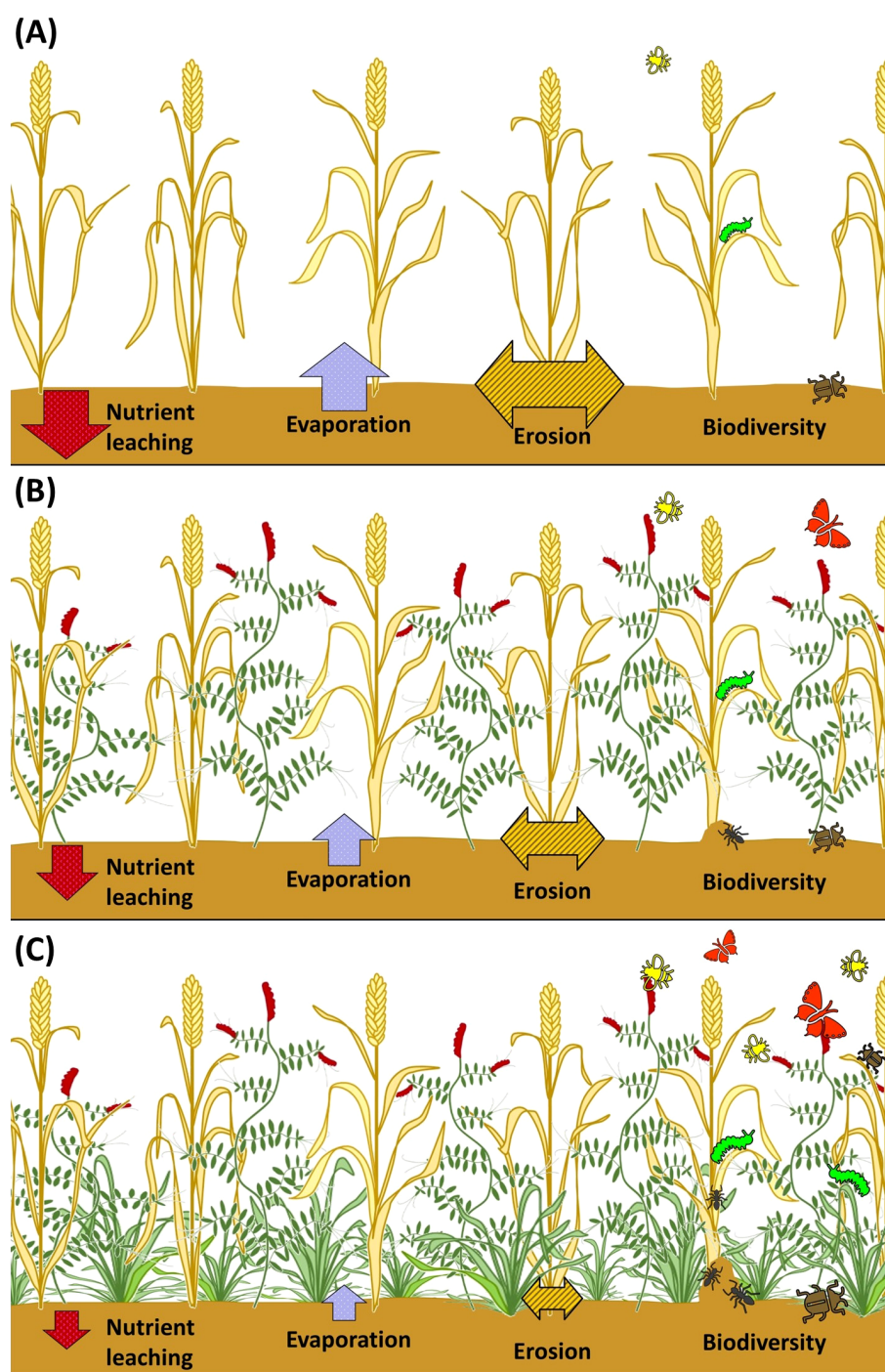


FIGURE 3

Schematic representation of an arable monoculture (A), a simple intercropping of two plant species (B), and an extended intercropping (mixed cropping) of more than two plant species (C). The size of the arrows indicates changes in nutrient leaching, evaporation and erosion (arrow size) due to the increase in plant diversity-related soil cover and soil rooting. The insects' number and size schematically represent changes in habitat conditions for faunistic biodiversity.

Raseduzzaman and Jensen (2017) investigated 33 articles to assess the grain legume intercropping effect on cereal yield stability. They found that across major climatic zones (Tropical Zone, Subtropical Zone, and Temperate Zone), cereal-grain legume intercropping significantly increased the yield stability compared with respective sole cropping systems.

Furthermore, the meta-analysis by Verret et al. (2017) investigating the effects of intercropping on weed suppression in cash crops (e.g., corn, forage) included 34 articles and encompassed 476 experimental units. Each unit represented a unique combination of factors like site, year, cash crop type, legume companion species, and agricultural practices. The analysis

showed that intercropping significantly reduced weed biomass by 56% compared to non-weeded monoculture control treatments.

The work of [Bedoussac et al. \(2015\)](#) investigated the effects of intercropping on grain yield, protein concentration, economic return, and resource utilization across 58 field experiments conducted in diverse European pedo-climatic conditions. The authors found that intercropping yielded higher and more stable grain yields compared to the average sole crop yield (0.33 kg m^{-2} vs 0.27 kg m^{-2}). In addition, intercropped cereals exhibited a higher and more stable protein concentration than sole cereals (11.1% vs 9.8%). Furthermore, intercropping resulted in a significant increase in gross margin compared to the average sole crop gross margin (702 € ha^{-1} vs 577 € ha^{-1}). Advantages in intercropping were observed due to likely better resource use, such as light interception efficiency and more balanced utilization of both soil mineral N and atmospheric N_2 fixation. Importantly, the overall advantages of intercropping were most pronounced in systems with low N availability. Similar findings were observed in an organic farming system in a semi-arid environment of southern Italy, where durum wheat and forage legumes produced higher grain yield and grain protein than durum wheat monocrop ([Scordia et al., 2024](#)).

3.1.3 Mixed varieties

Varietal mixing is an agricultural practice that consists of sowing a heterogeneous mixture of varieties of the same species in the same plot ([Figure 4](#)).

It has been reported that planting varietal mixtures leads to more stable yields, especially when faced with biophysical constraints such as droughts, erosion, poor nutrient contents, and heavy pest pressure or weed infestation ([Von Cossel et al., 2019](#)).

A meta-analysis examining over 3,600 observations from 91 studies ([Reiss and Drinkwater, 2018](#)) found a surprising benefit to planting multiple crop varieties together (intraspecific mixtures). These mixtures yielded 2.2% more on average compared to fields

planted with a single variety (monoculture). This advantage was even greater under stressful conditions, like low nutrients or heavy pest pressure. The authors also revealed that planting variety mixes led to more stable yields over time, especially when faced with year-to-year weather variations.

[Borg et al. \(2018\)](#) conducted a comprehensive review of 32 research studies examining wheat mixtures in comparison to their individual components grown in pure stands. Their analysis demonstrated a notable increase in yield of 3.2% for each additional component variety when disease pressure was high. Overall, the average yield increase observed was 3.5%, with this figure climbing to 6.2% under conditions of elevated disease risk. These findings strongly suggest that cultivating mixed varieties of wheat holds significant promise for enhancing crop yields, particularly in agricultural settings that prioritize reduced pesticide use.

Worth to mention is the review of [Hajjar et al. \(2008\)](#), who found that increasing crop genetic diversity in arable systems could help increase pollination services and soil processes (carbon sequestration and soil erosion mitigation), contributing to the long-term stability of agroecosystems. Potential drawbacks or consequences along the values chain were identified, such as heterogeneous quality, practical and economic implications for processing (harvest and sorting the harvested material) among others.

3.1.4 Cover cropping

Cover crops, i.e., unharvested crops grown together or between primary cash crops, are used for multiple objectives, ultimately improving soil health and enhancing yields ([Scavo et al., 2022](#)). A meta-analysis by [Garba et al. \(2022\)](#) examined the influence of cover crops ([Figure 5](#)) on cash crop yield, soil water content, and soil mineral N in dryland environments. The analysis encompassed 1006 observations for cash crop yield, 539 observations for soil water content, and 516 observations for soil mineral N. The study identified



FIGURE 4

Example of a winter wheat (*Triticum aestivum* L.) population mixture in an organic farm located in Patti (Messina, Italy). The picture shows the different morphology of winter wheat inflorescences (photo courtesy of Aurora Maio, from the experimental farm of the University of Messina).



FIGURE 5

Example of a cover cropping approach in viticulture. The site is in Rodi Milici (Messina, Italy, 100 m a.s.l.). The grape variety is “Nero d’Avola”, and the cover crop mix consists of *Vicia faba* var. Minor, *Trifolium alexandrinum*, *Hedysarum coronarium*, *Avena sativa*, x *Triticosecale*, and *Hordeum vulgare* (photo courtesy of Francesca Calderone, from the experimental farm of the University of Messina).

a minimum annual precipitation threshold of approximately 700 mm, acting as a “break-even point” for achieving significant yield benefits from cover crops compared to control fallows. Overall, cover cropping resulted in an average decrease of 7% in cash crop yield, 18% in soil water content, and 25% in soil mineral N. However, across climatic zones, soil types, and specific crop management practices, subsequent cash crop yields varied by +15%, +4%, -12%, and -11% in tropical, continental, dry, and temperate dryland climates, respectively. These findings highlight the importance of a thorough understanding of cover crop integration into cropping systems to minimize potential trade-offs between ecosystem services (e.g., soil health improvement) and disservices (e.g., reduced water availability for cash crops).

A meta-analysis by [Jian et al. \(2020\)](#) investigated the impact of cover crops on SOC, showing a significant increase (15.5% mean change) when cover crops were integrated into crop rotations. The mean rate of C sequestration attributable to cover cropping across all studies was $0.56 \text{ Mg ha}^{-1} \text{ yr}^{-1}$. The largest SOC increase was found in shallow soil layers ($\leq 30 \text{ cm}$), in fine-textured soils (39.5% mean change), followed by coarse-textured (11.4%) and medium-textured (10.3%). In temperate and tropical climates SOC raised by 18.7% and 7.2%, respectively. SOC further improved in cover crop mixtures than monoculture cover crops, and in legume cover crops than in grass species, and in species with higher biomass yield. Other soil quality parameters were enhanced, such as reduced runoff and erosion, and increased levels of mineralizable C, mineralizable N, and total soil N. Additional factors influencing SOC change were annual temperature, duration of cover crop implementation, geographic latitude, and initial SOC concentration.

The review of [Kaye and Quemada \(2017\)](#) highlighted that ecosystem services from using cover crops can synergistically

promote services related to climate change. They found that soil carbon sequestration and reduced fertilizer use after legume cover crops can mitigate approximately $100\text{--}150 \text{ g CO}_2\text{e m}^{-2} \text{ year}^{-1}$ of greenhouse gas fluxes, and the vegetation cover may mitigate 12 to $46 \text{ g CO}_2\text{e m}^{-2} \text{ year}^{-1}$ of surface albedo change over a 100-year time horizon.

3.1.5 Crop rotation

Crop rotation is the practice of planting different crops sequentially on the same plot of land. The global metaanalysis (11,768 yield observations from 462 field experiments) by [Zhao et al. \(2022\)](#) demonstrated that legume-based rotations have the potential to enhance crop production, especially when integrated into low-input and low-diversity agricultural systems (32%) than high-yielding environments (7%). Legumes, as pre-crops, consistently enhanced main crop yield (rice, wheat, maize) by 20% as compared to non-legume pre-crops across pedo-climatic regions.

[John et al. \(2021\)](#) found out that legume crop diversification in maize cropping, either in rotation system or intercropping groundnut, allowed for increased yield, protein, stability, and profits as compared to unfertilized and full fertilized maize monocrop across 29 farm sites (120 year-site combinations) in central Malawi (Africa). The legume diversification system performed best in marginal environments. The soil organic carbon was influenced by soil texture (sites with SOC $>1.5\%$ had sand content $<50\%$) rather than the legume diversification system. Despite these positive results, authors drew attention to the need for agricultural policies that increase access of farmers to superior legume seeds and agroecology-based advice.

The multilevel regression analyses of [Bowles et al. \(2020\)](#), demonstrated that across a precipitation gradient in continental

environmental zone of North America, more diverse rotations increased maize yields over time and across all growing conditions (28.1% on average). Even in drought years yield losses were reduced by 14.0%–89.9% under diverse rotation systems.

3.1.6 Mixed farming

Mixed farming involves crop-to-livestock integration on the same farm. Research by [Pent \(2020\)](#) analyzing 22 studies found that combining trees, pastures, and livestock in a single system (example see [Figure 1](#)), can significantly increase overall productivity. Compared to managing these elements separately, silvopastoral agroforestry practices can boost land output by 42–55%, depending on whether livestock production or forage yield is to be taken into account. Interestingly, this “overyielding” effect often occurs even when the individual production of trees, forage, or livestock goes down slightly within the silvopastoral agroforestry system. This suggests that the combined benefits outweigh any minor reductions in individual yields.

[Jordon et al. \(2022\)](#) carried out a meta-analysis with contradictory results on the overall sustainability of three selected agroecological practices (no-/reduced tillage, cover cropping, and ley-arable) in the temperate oceanic regions. The study found evidence (195 paired observations taken from 40 studies, most of them located in the UK, France, The Netherlands, Denmark, and Germany) for agroecological practices increasing the soil organic carbon but not the yield. They concluded that more research is needed on the question of how livestock can be best integrated to agroecological farming systems to create win-win opportunities for the farms, especially concerning the applications of ley-arable strategies. These recommendations are thus in line with those brought up by [Snapp et al. \(Snapp et al., 2023\)](#).

Research by [Falkowski et al. \(2023\)](#), who collaborated with Maya farmers (milperos) in several communities in the Montes Azules Biosphere Reserve region in Chiapas (Mexico) highlighted a surprising fact: the dynamic polyculture system full of genetic resources produces charcoal that retains carbon at a rate 4 to 14 times higher than slash-and-burn systems reported elsewhere. While burning releases significant carbon ($12.6 \pm 3.6 \text{ t C ha}^{-1} \text{ yr}^{-1}$), char production ($3.0 \pm 0.6 \text{ t C ha}^{-1} \text{ yr}^{-1}$) and incomplete combustion help offset some of this loss. Interestingly, burning had minimal impact on soil composition, but it did significantly increase pH, potassium availability, and cation exchange capacity (by 2%, 100%, and 7%, respectively). This study suggests that Maya milpas, with their unique char production and management practices, have the potential to become long-term carbon sinks. However, this benefit hinges on the preservation of ecological knowledge within Maya communities. Socioeconomic changes and the potential for shortened fallow periods or land tenure insecurity could threaten this sustainable practice.

The review by [Thornton and Herrero \(2014\)](#), who discussed adaptation options available to smallholders in mixed crop–livestock systems in developing countries is worth mentioning. Among potential mitigation co-benefits, improving feeding through diet supplementation and improved grass and fodder species ranked highest in their analysis. However, high costs,

labor demands, and lack of knowledge were identified as constraints to adoption. Other potential practices included the management of nutrients and soil, manure, grazing, and crop residues, with variable impacts on food security, resilience, and the promotion of diversification, along with managing risks (e.g., costs, competing demands, labor demand, limited access to information and technologies, lack of knowledge). They concluded that effective adaptation would require supportive policies, technical advancements, improved infrastructure, and better access to information, emphasizing that the development challenge remains significant and complex.

3.2 Soil management

To bolster the resilience of cropping systems, it is crucial to carefully consider the various tillage and amelioration practices that can be integrated into agroecological frameworks. These practices, when thoughtfully implemented, can significantly enhance soil health, improve water retention, and mitigate the adverse effects of climate change. Hence, the following section addresses specific tillage and amelioration techniques regarding their potential benefits and challenges for agroecological farming.

3.2.1 Tillage

Tillage, involving mechanical actions such as digging, stirring, and overturning, is the most common method used for soil preparation in agriculture. Conservation practices, such as reduced tillage, minimum tillage, and no-tillage, aim to preserve soil structure and health ([Altieri et al., 2017](#)) ([Figure 6](#)).

These practices focus on enhancing soil organic matter (SOM) by reducing soil degradation processes. A global study by [Huang et al. \(2018\)](#) examined the effects of no-till farming compared to conventional tillage. This analysis focused on greenhouse gas emissions (methane, carbon dioxide, and nitrous oxide), crop yields, and the overall impact on global warming for major cereal crops:

- Reduced methane emissions: No-till farming decreased methane emissions by an average of 15.5%.
- Increased nitrous oxide emissions: However, it also led to a 10.4% increase in nitrous oxide emissions, another greenhouse gas.
- Climate impact varies: The impact on crop yields depended on climate. No-till practices benefited yields in dry areas but hurt them in humid regions.
- Soil pH matters: On acidic soils, no-till reduced global warming potential without harming yields. Conversely, on alkaline soils, it increased yields without affecting global warming potential.
- Crops respond differently: Barley yields increased significantly (by 49%) with no-till, especially in dry climates. Rice fields also benefited, with a 22% reduction in both carbon dioxide and methane emissions. However, maize yields decreased.

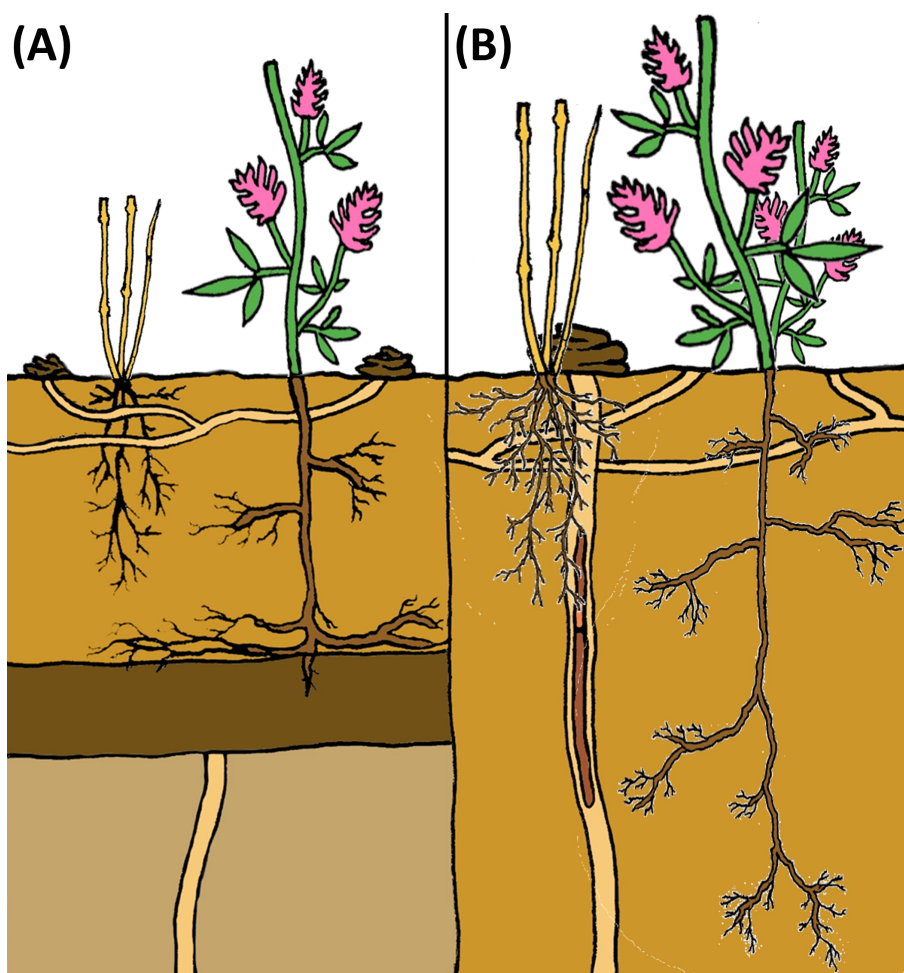


FIGURE 6

Schematic illustration of effects on the rooting zone of conventional tillage [(A), indicating a compacted layer at ploughing depth of about 25 cm depth] and no-till management [(B), indicating a higher earthworm activity and higher biomass growth and deeper rooting depth] (adapted from Hoeffner et al., 2022, and Pelosi et al., 2014). The brown soil casts on the soil surface represent the earthworms' excrement (small roll-shaped soil aggregates of clay-humus complexes), which are associated with mineral grains and plant remains and form a loose pile of smaller crumbs.

Overall, the effectiveness of no-till depends on several factors, including climate, soil characteristics, and crop type (Huang et al., 2018). Therefore, farmers need to consider their specific environment when choosing tillage practices. The authors also found that combining no-till with reduced N fertilizer rates can increase crop yields without worsening greenhouse gas emissions. Additionally, it was recommended exploring subsurface placement of N fertilizers in no-till systems to further reduce nitrous oxide emissions.

A long-term, 36-year study conducted in a temperate region examined the impact of crop rotation diversity and no-till cultivation on maize drought resilience. Surprisingly, the findings indicate that no-till practices did not influence the maize plants' ability to withstand drought conditions (Renwick et al., 2021). However, further analysis through path modeling confirmed a robust association between increased SOM and decreased water stress in maize plants, even though there were no measurable differences in SOM levels among the various crop rotations or tillage methods nor higher soil water retention, infiltration, or

differential root water depth, suggesting that other mechanisms require investigations.

Lal (2020) also approved that increasing SOM content enhances plant-available water across all soil types (sandy, silty, and clayey textures) and can contribute to drought resilience by conserving water resources. As expected, the magnitude of this increase depends on site-specific inherent and external factors. This effect is attributed to a relatively greater increase in field capacity compared to the wilting point. Further research is needed to better understand the mechanisms and soil processes that lead to increased plant-available water content in relation to higher SOM levels.

3.2.2 Organic farming

Organic farming is aimed at avoiding or largely excluding (depending on the underlying certification requirements) the use of synthetic compounds, such as chemical fertilizers, pesticides, herbicides, growth regulators and livestock feed additives throughout agricultural practices. This common goal makes organic farming and agroecological farming similar, although

agroecological practices are not necessarily applied in organic farming. Ponisio et al. (2015), meta-analyzed organic and conventional yields with more than 1000 observations. Overall, it was found that organic yields were only 19.2% lower than conventional yields, with different effects of crop types and management practices on the yield gap. The yield gap between organic and conventional monocultures was $17 \pm 3\%$ and increased to $21 \pm 6\%$ in organic and conventional polycultures. When organic and conventional did not include crop rotation, the yield gap was $16 \pm 5\%$, while it increased to $20 \pm 2\%$ when both systems had a similar number of rotations. The most affected crops were root and tuber, with yield reduction of $30 \pm 11\%$, followed by cereals ($22 \pm 3\%$), vegetables ($17 \pm 4\%$), legumes ($15 \pm 10\%$), oilseed ($13 \pm 5\%$), fruit and nuts ($7 \pm 5\%$). The authors underscored the importance of strategic investments in agroecological research as a means to enhance organic farming practices. Such investments, they suggested, could potentially bridge or entirely close the yield gap for certain crops or in specific geographic areas.

A rigorous assessment by Knapp and van der Heijden (2018) examined the year-to-year consistency of crop yields across three primary agricultural systems: organic farming, conservation agriculture, and conventional agriculture. The study, which drew on data from 193 studies and 2896 observations, accentuates that organic agriculture exhibits a notably lower degree of yield stability, with a 15% decline in consistency per unit of yield compared to conventional farming. While organic farming undoubtedly contributes to biodiversity and environmental sustainability, future research and development efforts should prioritize strategies to mitigate its inherent variability in crop yields. The authors suggest that incorporating green manure and optimizing fertilization practices could help narrow the gap in yield consistency between organic and conventional agriculture. Furthermore, the analysis uncovered that adopting no-till techniques within conservation agriculture does not significantly impact yield stability, as evidenced by its temporal stability of -3%, which is comparable to that of conventional tillage methods. This finding implies that transitioning to no-till farming does not compromise the consistency of crop yields.

3.2.3 Mulching

Mulching is a practical and affordable agricultural practice that can be readily implemented by farmers. This technique involves covering the soil surface with organic or inorganic materials to enhance soil structure, retain moisture, regulate soil temperature, and minimize nutrient loss, salinity, and erosion (Iqbal et al., 2020). The origin of the mulch material (on-farm or off-farm) strongly depends on the intended mulching effects (e.g., high or low albedo effect) and the local conditions (farming system, other farms in the region, seasonal straw yields/prices etc.) (Iqbal et al., 2020).

A thorough meta-analysis by Qin et al. (2015) investigated the effects of mulching on wheat and maize production, drawing on a vast dataset of 1310 yield observations from 74 studies conducted

across 19 countries. The analysis indicated that mulching significantly enhanced yields, water use efficiency (WUE), and Nitrogen use efficiency (NUE) by up to 60% compared to non-mulched crops. These benefits were more pronounced in maize than in wheat and were more substantial when plastic mulch was used instead of straw mulch. Notably, plastic mulch proved more effective in relatively cool conditions, while straw mulch exhibited the opposite pattern. Additionally, the benefits of mulching tended to diminish as water availability increased. The positive effects of mulching were not influenced by the organic matter content of the soil. The authors concluded that mulching can play a crucial role in bridging the yield gap between potential and actual crop yields, particularly in arid regions and agricultural systems with limited nutrient inputs. However, the management of soil mulching requires site-specific knowledge.

Fraga and Santos (2018) conducted a modeling study to predict grape yields in the Alentejo wine region under the RCP8.5 climate change scenario over the next 60 years, comparing non-mulched and mulched vineyards (Southern Portugal). Authors found a general yield decline in grape yield due to warmed growing seasons, however, mulching can reduce the yield decreasing trend from $-0.75\% \text{ year}^{-1}$ in non-mulching to $-0.66\% \text{ year}^{-1}$.

3.2.4 Green manure

Green manure is undecomposed organic material (green) that can be obtained either by growing short-term crops (cover crops including legumes) and incorporating them into the soil in the same place (*in-situ*) or by collecting green leaf residues (*ex-situ*) from nearby sources and integrating them into the soil a few days (15-30) before sowing the main crop (Meena et al., 2018).

An in-depth meta-analysis conducted by Ma et al. (2021) evidenced that the application of green manure in Northern China significantly enhanced soil health. Key benefits included a reduction in soil bulk density by approximately 5.6%, a 28% increase in microbial biomass carbon, and a 14-39% improvement in soil enzyme activity. Among various green manure types, legume-based green manure more effectively increased nitrate and hydrolyzable N levels, while non-legume green manure more notably elevated available potassium. Although green manure treatment led to a decrease in soil gravimetric water content, it consistently boosted maize yields by 11% on average. However, the impact of green manure on wheat and potato yields was less predictable. In conclusion, the strategic use of green manure in Northern China offers a promising avenue for improving soil quality and enhancing cash crop production. For example, a field study on several forage legumes in Maragheh (Iran), such as, among others, grasspea (*Lathyrus sativus*), maragheh vetch (*Vicia villosa*), berseem clover (*Trifolium alexandrinum*) and sanfoin (*Onobrychis sativa*) showed that across species, green manure had significant effects on SOC, calcium carbonate equivalent (CCE), bulk density, moisture percentage and electrical conductivity of soil extract (Habibi et al., 2013).

3.3 Holistic views on ecosystem services performance of agroecological farming

Jeanneret et al. (2021) explored the application of landscape ecology methods in agroecology, focusing on biodiversity conservation, regulating ecosystem services (pest control, pollination), agroforestry implementation, and agroecological innovations in a European context. Their mindset aligns with Altieri et al. (2015), and Morizet-Davis et al. (2023), emphasizing the crucial role of biodiversity in tackling future climate change challenges. In a thoroughly prepared review, Jeanneret et al. (2021) provide a wide range of relevant solutions and next steps to be taken toward a successful incorporation and upscaling of agroecological practices in European agricultural systems. The authors recommend that a better understanding of the potential benefits of traditional agroecological farming on ecosystem services requires a site-specific bottom-up assessment. This approach should tailor the evaluation to the unique conditions and challenges of each location. Further research and involvement of the farmer's experiences and ideas are seen as crucial to identifying optimal combinations and scaling strategies for agroecological practices at the landscape level, maximizing their support for biodiversity and other ecosystem services.

Cadel et al. (2023) investigated the effects of maximizing ecosystem services (bundles) through agroecological practices on agricultural productivity. Since there are no significant effects of soil-based ecosystem services on agricultural production, it is possible to adopt agroecological practices without compromising the economic performance of the agricultural system, argued the authors. Key agroecological practices are (i) the implementation of wide and diverse rotations, (ii) the targeted use of cover crops, (iii) a reduction of tillage intensity, and (iv) a sound recycling of organic material by the application of organic fertilizers. According to Cadel et al. (2023), a more comprehensive review of further literature is recommended since only 40 documents are included in this meta-analysis. For instance, South America, Russia, and Africa are not covered by this study, indicating, but not proving, a potential lack of information on agroecological approaches in those regions. As a solution, authors suggest widening the view on literature by excluding search terms like “ecosystem services”.

Snapp et al. (2023) carried out a meta-analysis of 138 scientific articles selected from a total of about 30,000 articles, as well as several interviews with organizations. With climate change adaptation in focus, Snapp et al. found significant evidence for agroecological practices associated with farm diversification along with the co-creation of knowledge being most helpful in low- and middle-income countries to better cope with the ongoing climate crisis. Especially, wide crop rotations and the application of cover cropping strategies provide numerous positive impacts in terms of crop yield, pollination, pest control, nutrient cycling, water regulation and soil fertility. In contrast, there was only modest evidence for the potential climate impact of agroecological practices themselves. It was only found that agroforestry in the tropical zone could have a positive impact by sequestering atmospheric carbon in the soil. Hence, it was recommended to gather more information on the potential greenhouse gas emissions through the application of

agroecological practices. Further, according to Snapp et al., more data is required about livestock integration into agroecological farming systems, as well as the resilience of agroecological farming systems to extreme events.

An analysis of 15 case studies explored the impact of agroecology on food security and nutrition across four key areas: crop diversity, mixed farming with livestock, soil management, and socioeconomics (Bezner Kerr et al., 2021). Encouragingly, 13 out of the 15 cases showed positive outcomes, and it was shown that the combination of different agroecological practices, and especially also social innovations, increased the effect. While Bezner Kerr et al. (2021) provide strong evidence for the benefits of agroecology, the researchers acknowledge the need for more rigorous research designs. This includes methods like case-control studies and longitudinal studies, which can better isolate the impact of agroecology from other factors that influence food security and nutrition. Additionally, the study highlights the need for more research on the social and economic aspects of agroecology. This could include examining the role of direct marketing, addressing social inequalities, and improving land and natural resource governance.

Research by Himmelstein et al. (2017) across Africa found that intercropping boosted crop yields by an average of 23% and increased farmer income by \$172 per hectare. However, the effectiveness of intercropping varied depending on how it was managed and the local environment. Interestingly, the authors did not find a clear benefit from using legumes, reduced tillage, pesticides, or fertilizers in conjunction with intercropping. Additionally, while integrated pest management (IPM) alone increased yields by 20%, combining IPM with intercropping resulted in lower yields (24% less) than IPM alone. These findings suggest that intercropping is a promising approach for sustainable agriculture in Africa, but it's crucial to consider other factors for optimal results. One key factor is controlling weeds that compete with crops. The study highlights the need for further research to explore how intercropping interacts with other sustainable practices in different environmental and economic settings. This will help to refine intercropping techniques and maximize its benefits for African farmers.

A meta-analysis by Morugán-Coronado et al. (2020) investigated the effectiveness of several sustainable farming practices as alternatives to conventional monoculture systems. The study examined 187 experiments from 46 scientific publications. These sustainable practices included planting a variety of crops together (crop diversification), minimizing soil disturbance (conservation tillage), and using organic fertilizers. All these practices increased the amount of SOC. Notably, the most significant increase in SOC was observed with the integration of permanent alley cropping systems. Soil N levels followed a similar pattern to soil organic carbon (SOC), although no-tillage did not significantly affect N levels compared to conventional tillage. While soil phosphorus (P) content remained relatively unchanged, permanent alley cropping had a negative impact on P levels. Surprisingly, the presence of alley crops, conservation tillage practices, or organic fertilization did not significantly influence tree crop yields. However, annual crop yields were more sensitive to regional climatic conditions, potentially declining in warm and dry areas. In conclusion, the integrated

implementation of intercropping, conservation tillage, and organic fertilization effectively enhanced soil quality and fertility, while providing year-round ground cover to safeguard the soil. Morugán-Coronado et al. (2020) therefore suggested prioritizing annual alley cropping with minimum tillage over permanent crops with no-tillage, particularly in warm and dry regions, to mitigate potential negative effects on soil P and N availability. Furthermore, it was indicated that the assessed soil properties may not be the primary drivers of long-term variability in crop yield.

3.4 Isolated views and experimental approaches of applying agroecological practices

Lu et al. (2022) focused on the agroecological practices ‘conservation tillage’ and ‘cover crops’, in a meta-analysis based on about 30 studies from the US using a sign test approach by Bushman and Wang (2009). This systematic analysis identified several key factors influencing farmers’ decisions to adopt these agroecological practices, including their willingness to seek and utilize information, the size and vulnerability of their landholdings, and higher levels of income and formal education. However, this study does not specifically consider the agroecological farming concept which omits the contextualization of agroecological practices employed. Additionally, the study omits recommendations for further research on how these agroecological practices contribute to enhanced farm resilience. Further, given the absence of an agroecological focus in the study by Lu et al. (2022), replicating the investigation of driving factors for implementing more resilient agroecological practices in relation to farmer perceptions within agroecological contexts could be a valuable future research direction.

Christel et al. (2021) screened 100 scientific documents in search of evidence on the influence of entire farming concepts (conventional, organic, biodynamic) on the ecological quality of the soil. Literature was analyzed with a view on the respective sum of the cultivation concept-typical farming practices – not the individual practices. The term “resilience” is not mentioned directly, but it can be assumed that it is considered implicit in the biological functioning of the soil. Not surprisingly, the literature also shows that organic and biodynamic cultivation concepts have far more positive effects on the ecological quality of the soil than the conventional cultivation concept. It was also shown that large parts of Africa, and Eurasia are underrepresented in the number of scientific studies on the topic compared with the Americas. Following Christel et al. (2021) it can be recommended that organic fertilization and longer crop rotations are the most favorable practices to improve organic soil quality, and more studies on the influence of soil-conserving agricultural practices on the soil fauna are needed.

Regarding biological plant protection, a meta-analysis by Tonhasca and Byrne (1994) examined 21 studies on agroecosystems with diversified cropping systems. The analysis established that these diversified systems when compared to simpler control systems, harbored moderately lower populations of herbivorous insects. This

can help reduce the need for artificial interventions in the agroecosystem, which can enable more environmentally friendly cultivation of the plants compared to large-scale cultivation.

Another meta-analysis of 43 studies also found evidence that increased habitat diversity, such as more finely structured agricultural landscapes with wide crop rotations and the use of cover crops, leads to a greater supply of biocontrol agents (predators), which can reduce the need for plant protection measures (Langellotto and Denno, 2004).

4 Conclusions

Taken together, the meta-analyses reviewed in this study highlight the complex interplay among soil, plant, climate, management, and socio-economic context within the selected agroecological practices and their potential effects on the resilience of farming systems. Positive, null, or negative effects were identified in the different studies, which largely depended on the factors mentioned above.

In the agroforestry practice, common recommendations were the need for further research on (i) the overall benefit agroforestry can provide for more resilient farming systems at the field and landscape level, (ii) other companion planting options and designs, (iii) tree traits and diversity, (iv) crop varieties with tolerance to shade, along with (v) long-term monitoring to assess the whole lifespan of these systems. Careful consideration of these factors is essential to optimize crop yields and maximize the overall benefits of integrating trees into agricultural landscapes. In the best case, agroforestry can serve as a key measure in agroecological farming to increase the resilience of the system, for example by improving (i) erosion control potential, which helps to reduce soil degradation potential, (ii) habitat functioning, which helps to counteract the loss of biodiversity in agroecosystems, and (iii) response diversity, which improves the ability of the agroecosystem to recover from disturbances such as drought, flooding or pest infestation.

Less prominent but still important, cover crops in crop rotations can also strengthen the resilience of the farming system by increasing the soil’s organic carbon content and improving several soil chemical parameters. Furthermore, they increase the potential of the cropping system to act as a sink for atmospheric CO₂. However, this is a long-term process (approx. 150 years until saturation) (Poeplau and Don, 2015), the extent of which varies considerably depending on the climate and available water content of the soil, soil type, type of cover crop and duration, biomass yield and C/N ratio, as well as the initial SOC concentration.

Also, the net effect of no-till, relative to conventional tillage, was influenced by several environmental and agronomic factors (climatic conditions, tillage duration, soil texture, pH, crop species), which further emphasizes careful planning and improved knowledge of the interaction among crop, site-specific conditions, and management.

Intercropping integrated with pest management penalized crop yield more than the system alone, suggesting that effective implementation of intercropping would depend on considering

adequate control of competing vegetation. On the contrary, other studies proved that intercropping significantly reduced weed biomass, stabilized crop yield over time, and increased grain protein concentration and farm gross margin, with larger advantages under low levels of soil N availability and marginal settings, and in systems where the use of synthetic products are largely avoided.

Studies have shown that including legumes in agricultural systems, either as cover crops or intercropped with other plants or in rotation, can be a sustainable practice. Legumes make it possible to use more natural sources of N in agroecosystems, thus reducing the need for external fertilizers. Planting a variety of crop genotypes together (varietal mixtures) helps stabilize yields, especially under abiotic (droughts) and biotic stresses (heavy pest pressure or weed infestation) or poor nutrient soils. In the long-term, this helps improving the resilience via increased soil fertility which allows for a higher response diversity within the soil fauna.

Mulching is a promising agroecological practice to increase crop yields, WUE, and NUE, however, the management of soil mulching requires site-specific knowledge. Green manure generally improves soil quality, nonetheless, results on some crops (i.e., wheat and potato) were inconsistent as compared to others, like maize.

To ensure or even improve the long-term resilience of farming systems in the face of worsening climate change impacts, increased investment in agroecological research is crucial. This research should focus on four key areas:

- Bridging the yield gap: Organic management practices need improvements to close the yield gap between organic and conventional agriculture.
- Livestock integration: Research on effectively integrating livestock into agroecological systems (e.g., silvopastoral agroforestry) would be a useful step in creating win-win scenarios for farms, boosting both productivity and resilience.
- Complex multi-trophic systems: Developing and understanding more complex systems that integrate plants and animals across multiple trophic levels (feeding relationships) is essential. These systems can promote nutrient cycling and align with the principles of a circular economy, where resources are reused and waste is minimized (Lewandowski et al., 2024).
- Optimal agricultural and food policy conditions and regulations: Farmers are already confronted with a great deal of red tape in many places. It is therefore necessary to support farmers at the local level in integrating agroecological practices through a legal framework that is both worthwhile and easy to implement.

In conclusion, enhancing biodiversity at the field level, including macro-, meso-, and microflora and -fauna, through targeted agronomic practices to enhance crop diversification and ecological soil management has proven essential in the short term for driving the transition toward more agroecological and resilient farming systems. By fostering diverse biological interactions, these practices improve soil health, crop yield and stability, nutrient cycling, pest regulation, and overall ecosystem stability. This

approach not only enhances immediate agricultural sustainability but also lays the foundation for long-term resilience to climate variability and environmental pressures.

Author contributions

MC: Conceptualization, Investigation, Methodology, Visualization, Writing – review & editing, Funding acquisition, Supervision, Writing – original draft. DS: Conceptualization, Investigation, Methodology, Visualization, Writing – review & editing, Writing - original draft, Funding acquisition, Supervision. MA: Investigation, Writing – review & editing. FG: Conceptualization, Funding acquisition, Investigation, Methodology, Supervision, Writing – review & editing.

Funding

The author(s) declare that financial support was received for the research and/or publication of this article. This study was partially funded by the University of Hohenheim and the University of Messina.

Acknowledgments

The authors acknowledge the use of Gemini, a comprehensive language model from Google AI to improve the linguistic quality of the manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fagro.2025.1495846/full#supplementary-material>

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