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Root plasticity and interspecific complementarity improve water use efficiency and productivity in wheat-alfalfa intercropping

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Abstract

Background and aims

Intercropping of grain crops with pastures while having yield advantages, also alleviates environmental issues through the optimal use of resources, improving sustainability. The mechanisms regulating water uptake and yield formation under intercropping, particularly, the root plasticity and interspecific complementarity remains unclear. This study aimed to elucidate the mechanisms controlling yield responses of wheat and alfalfa, and to investigate interspecific root interactions and their effects on water use efficiency and productivity in both crops.

Methods

A three-year field experiment was carried out on the Loess Plateau with three cropping patterns: sole wheat (SW), sole alfalfa (SA), and wheat-alfalfa intercropping (W/A). Two nitrogen application rates were implemented, including conventional nitrogen (HN) and reduced nitrogen (LN). The wheat-alfalfa intercropping system was arranged with two rows of wheat intercropped with one row of alfalfa, in accordance with locally recommended planting ratios.

Results

Intercropping of wheat and alfalfa promoted root system growth and proliferation in both crops, mainly through increases in root biomass, root length density, root volume and root surface area during the co-growth treatment under both nitrogen application rates. The increased root system growth under intercropping enhanced soil water uptake, resulting in an increase in WUE_B of 15.5% and 17.1% under HN and LN, respectively. Alfalfa forage yield increased by 34.0% and 22.9% under HN and LN, while wheat grain yield did not change significantly in the first growing season of the experiment but increased by 24.6% and 25.9% under HN and LN application rates in the second growing season. Wheat-alfalfa intercropping had a high land equivalent ratios (LER) of over 1.30. The intercropping system produced 9.5–53.6% higher net income than the SW.

Conclusions

Wheat-alfalfa intercropping improved WUE_B and crop productivity through enhanced root growth and proliferation of both crops, and achieved high land equivalent ratio and net economic benefits compared with sole wheat cropping. This suggests that wheat-alfalfa intercropping represents an efficient and sustainable cropping strategy for the Loess Plateau, with favorable ecological and economic performance.

Highlights

Wheat/alfalfa intercropping increased root biomass, root length density and root volume of both crops.

Intercropping improved the system water use efficiency by strengthening root systems.

Wheat/alfalfa intercropping can improve crop productivity and land equivalent ratio in semiarid regions.

Wheat/alfalfa intercropping may be one of the solutions for food security and environmental sustainability.

1. Introduction

In the semiarid regions of the Loess Plateau in China, long-term monoculture of grain crops has accelerated soil nutrient depletion, resulting in soil infertility, degradation and secondary salinization, posing a great threat to efficient and sustainable agricultural production (Yin et al., 2020; Zhang et al., 2022). Long-term monoculture of leguminous forage also causes nutrient imbalance and food insecurity (Mu et al., 2023). Leguminous forage, such as alfalfa, is a perennial meadow that lasts 4 to 5 years and can be rotated with cereals to provide good productivity of both crops. Therefore, the transition from single grain or single leguminous forage cultivation to grain/forage intercropping seems to be a promising agricultural practice in order to improve agricultural affordability and food security (Stratton et al., 2022; Ma et al., 2023). Intercropping of cereals and legumes is an effective solution to alleviate the problems caused by intensive farming, as it improves soil fertility while meeting food demand (Kermah et al., 2017; Ma et al., 2024). Cereal-legume intercropping can also ensure crop yields while providing high-quality forage for livestock (Ma et al., 2024).

The Loess Plateau is an ecologically fragile environment in China and a major agricultural production region (Ma et al., 2024). The loose structure of loess soil, combined with low topsoil water holding capacity and poor water infiltration, results in weak water and nutrient retention, thus exacerbating soil erosion and leading to low soil organic matter and nitrogen contents (Jin et al., 2018; Ma et al., 2024). Long-term monoculture of wheat, maize or forage further exacerbated environmental degradation on the Loess Plateau (Ali et al., 2021; Ma et al., 2024). Therefore, changing the current long-term monoculture state is of great significance to alleviate land use conflicts in these regions. Wheat-alfalfa intercropping, as a new cropping design, was proposed to cope with water shortages, soil erosion, and insufficient grain and forage production (Kermah et al., 2017; Mu et al., 2023; Ma et al., 2024). Alfalfa is a crop with significant biological N-fixation ability, which can alleviate the problems of infertile soil and dependence on synthetic fertilizer application caused by long-term wheat monoculture (Sun et al., 2018). Alfalfa also has a well-developed and deep root system, which can loosen the soil, increase the organic matter content, and improve the soil structure, thereby benefitting the growth and development of crops (Kanwar et al., 2005; Li et al., 2007). Intercropping alfalfa with other crops can improve light, water, nutrients and land utilization, reduce pests and diseases, and suppress weeds (Zhang et al., 2011; Jungers et al., 2021; Li et al., 2007). Therefore, intercropping alfalfa with grain crops not only ensures the security of livestock feed, but also provides a promising and sustainable option for the Loess Plateau agricultural system.

Underground root system interactions between crops are more significant than aboveground crop interactions, and root growth and distribution are crucial for interspecific interactions in intercropping systems (Wilson, 1988; Wu et al., 2012). Under intercropping, the complementary effect is more marked when root distribution and water uptake patterns of the two crop species are different (Mu et al., 2023). Root extension under intercropping can further promote root growth (Bargaz et al., 2015) by increasing soil pore space (Simbine et al., 2018), thereby improving soil water use efficiency (Yin et al., 2020) and nutrient use efficiency (Ma et al., 2024). For instance, wheat-soybean intercropping has higher root biomass and root length density than their monoculture counterparts, because the roots of the two intercropped crops penetrate each other and fully utilize underground resources (Ren et al., 2017). However, intercropping may also alter root distribution and limit growth (Liu et al., 2020). Under the maize-soybean intercropping, root development of soybean is restricted (Gao et al., 2016). N availability plays an important role in regulating

root system complementarity and/or competition under the cereal/legume intercropping (Zhang and Li, 2003). For instance, in the cereal/legume intercropping, low nitrogen input will intensify root competition and increase yield through the facilitative effect of competition, while excessive N supply will exacerbate the inhibitory effect, risking crop productivity and environmental sustainability (Hu et al., 2016). These findings highlight the critical role of root traits and nitrogen management in mediating interspecific interactions in cereal-legume intercropping, yet such mechanistic insights remain poorly explored in the specific system of wheat-alfalfa intercropping.

Although enhanced water use efficiency has been observed in wheat-alfalfa intercropping in previous studies, the underlying mechanisms by which root plasticity, root distribution and interspecific root complementarity modulate this improvement remain largely unclear. Moreover, most existing research has focused on aboveground plant traits rather than root system responses, despite the well-documented key role of root growth and proliferation in determining crop productivity and water use efficiency under intercropping conditions (Mu et al., 2023; Ma et al., 2024). Based on the above problems, this study conducted a 3-year field experiment to (1) examine the root growth and vertical distribution of wheat and alfalfa and clarify their interspecific competition and complementarity; (2) evaluate the synergistic effects of root plasticity and temporal niche differentiation on water a use efficiency; (3) verify the productivity and economic benefits of the intercropping system under different nitrogen application. We hypothesized that the yield advantage of wheat-alfalfa intercropping is driven by root plasticity and temporal niche differentiation, and root complementarity is the key underground mechanism for efficient resource utilization under temporal differentiation.

2 Material and Methods

2.1 Experimental site

A three-year field study covering two winter wheat growing seasons was conducted from March 2021 to June 2024 in Yangling, Shaanxi Province, China (34°16'N, 108°04'E; 460 m asl). The climate of the test site is semi-arid and semi-humid, with a total annual precipitation of 635 mm (577 mm in 2021, 587 mm in 2022, and 635 mm in 2023), a potential evaporation of 993 mm and an average annual temperature of 12.9℃ (Fig. 1). The soil of the experimental field was loess, with a bulk density of 1.20 g cm^{-3} and a field capacity of 24% (q/v). The average nutrient content of topsoil (0–20 cm) was: soil organic matter 11.6 g kg^{-1}; total N 1.1 g kg^{-1}; total phosphorus 0.7 g kg^{-1}; available phosphorus 20.6 mg kg^{-1}.

2.2 Experimental design and field management

The field experiment was a split-plot design with three planting patterns and two N fertilization levels. Planting pattern was the main plot factor, and two N application rates, randomized into each main plot. The three planting patterns were sole wheat (SW), two rows of wheat intercropped with one row of alfalfa (W/A), and sole alfalfa (SA) (Fig. 2). The arrangement of wheat and alfalfa in the intercropping system was set based on the locally recommended intercropping ratio. Two rates of N fertilization, conventional nitrogen (HN, 150 kg N ha^{-1}) and reduced nitrogen (LN, 75 kg N ha^{-1}), were used in the study. The N

fertilization rates for wheat were set based on the N-fixation capacity of alfalfa, with the low rate of 75 kg N ha^{-1} being close to the lower limit of that capacity (Wang et al., 2019). This approach aimed to verify whether crop N demand could be met by reducing external N input and relying on biological N-fixation. The rate of 150 kg N ha^{-1} was set as the upper limit of alfalfa N-fixation. In both cases, N was applied as urea, and P was applied as superphosphate at a uniform rate of 120 kg P ha^{-1} to all plots. Alfalfa received 90 kg N ha^{-1} under the HN treatment and 45 kg N ha^{-1} under the LN treatment, along with 120 kg P ha^{-1} before sowing in 2022. In 2023 and 2024, 50 kg P ha^{-1} was applied before regrowth. The N application rates for alfalfa (90 kg N ha^{-1} for HN and 45 kg N ha^{-1} for LN) were used to test whether soil N supply affects the growth response and N-fixation efficiency of alfalfa. No potassium fertilizer was applied because soil tests indicated sufficient K availability.

Winter wheat (*Triticum aestivum* L. var. Changhan 58) was planted on 10 October 2022 and 11 October 2023 and harvested on 29 May 2023 and 31 May 2024. Alfalfa (*Medicago sativa* L., Handi) was sown on 5 April 2022, and harvested in October 2022, and additional cuts were made in April, June and October of 2023, and April and June in 2024 (Fig. 1). Harvest time of alfalfa were made at early flowering, when leaf nutrient content is high, and the indigestible components such as cellulose are relatively low (Mu et al., 2023). The plot area was 9 m^2 (3 m × 3 m) with 1.0 m space between plots and containing four ridges. The sown rate of wheat was 150 kg ha^{-1} with row space of 20 cm, while alfalfa was sown at a rate of 22.5 kg ha^{-1} with row spacing of 25 cm. In intercropping plots, wheat crops were spaced 20 cm apart, and wheat and alfalfa were intercropped at 25 cm apart (Fig. 2). Irrigation was no provided. Manual weeding was carried out at jointing and grain filling stages of wheat and one month before the second and third alfalfa harvest.

2.3 Root system growth and distribution

In order to evaluate the vertical and horizontal root distribution and morphology of wheat and alfalfa under mono-cropping and intercropping, the root sampling points (P) were P1 and P2 for sole wheat, P4 and P5 for sole alfalfa, and P1-P4 for intercropping of wheat and alfalfa at anthesis (Zadoks 63) and physiological maturity stages (Zadoks 91) of wheat (Fig. 2). Root system samples were collected from 0-200 cm soil profile using a dormer auger with 9 cm diameter. The root samples were divided into four layers: 0–20 cm, 20–60 cm, 60–120 cm and 120–200 cm. The collection and classification of root samples are based on Chinese soil classification and root characteristics of wheat and alfalfa (Guo, 1992; Kanwar et al., 2005; Li et al., 2024). The roots were immediately and gently washed to remove the soil through a 2 mm sieve. The roots of wheat and alfalfa were distinguished mainly by their differences in morphology and presence of nodules. Wheat roots were light yellow, and alfalfa roots were brown and often had nodules. After washing, root images were analyzed using WinRHIZO (Regent Instruments Inc., QC, Canada) to measure root length (RL), root surface area, and root volume (V). Root length density (RLD) was calculated according to the following equation:

$$\mathrm{RLD}=\mathrm{RL}/\mathrm{V}$$

After the scanning was completed, the roots were dried in a forced-air oven at 80°C for 48 h to determine root biomass.

2.4 Soil moisture

Before sowing and at anthesis and maturity of wheat, soil samples were collected using a 9 cm diameter soil auger to determine gravimetric soil water content. Soil water content was determined in 0-200 cm soil profile, and it was divided into 10 layers of 20 cm each, with three replicates per layer.

2.5 Wheat grain yield, alfalfa forage yield and water use efficiency

In monocropping plots, three rows of one-meter-long each were randomly selected and sampled to determine wheat grain yield and alfalfa forage yield. In intercropping plots, three strips of one meter each of wheat and alfalfa were also harvested to determine wheat grain yield and alfalfa forage yield. The number of wheat spikes in each sample was counted and used to determine the spike density. Prior to threshing the spikes, 20 spikes were selected to determine the average number of grains per spike. The wheat grains were threshed, dried in an oven at 70°C to constant weight, and weighed. The 1000-grain weight of wheat was obtained by randomly counting 1000 grains. For alfalfa, fresh weight was measured, and the samples were dried at 105℃ for 30 minutes to cease biological activity, then dried at 75℃ to constant weight, weighed and hay yield was calculated. Water use efficiency (WUE_B) of biomass was calculated as the ratio of aboveground biomass to evapotranspiration:

$$\mathrm{WUE_B}=\mathrm{AB}/\mathrm{ET}$$

Evapotranspiration (ET) was determined as follows:

$$\mathrm{ET}=\mathrm{P}+\Delta\mathrm{W}$$

where P is rainfall during the crop growing season, and ΔW is the difference of soil moisture stored in 0-200 cm soil profile between planting and maturity.

2.6 Interspecific competition

Aggressiveness (Apm) is used to quantify the interspecific competition, reflecting the degree to which one crop competes with the other under intercropping (Banik et al., 2000). Apm was determined as follows:

$$\mathrm{Apm}=\frac{\mathrm{Y_{IA}}}{(\mathrm{Y_{SA}}\times\mathrm{P_A})}-\frac{\mathrm{Y_{Iw}}}{(\mathrm{Y_{SW}}\times\mathrm{P_W})}$$

where Apm is the competitive ratio of alfalfa relative to wheat. Y_{IW} and Y_{IA} are the aboveground biomass of wheat and alfalfa in intercropping, while Y_{SW} and Y_{SA} are the aboveground biomass of wheat and alfalfa in monoculture, respectively. PW and PA are the proportions of wheat and alfalfa in intercropping system, respectively. Positive Apm values indicate that alfalfa has a competitive advantage over wheat in intercropping systems (Ma et al., 2024).

2.7 Land equivalent ratio, actual yield loss and economic benefit

Land equivalent ratio (LER) is a measure of land productivity used to evaluate the use of land resources in intercropping systems (Li et al., 2006). LER was calculated as follows:

$$\mathrm{LER}=\frac{\mathrm{Y_{IW}}}{\mathrm{Y_{SW}}}+\frac{\mathrm{Y_{IA}}}{\mathrm{Y_{SA}}}$$

where Y_{IW} and Y_{IA} are the grain yield of wheat and forage yield of alfalfa in the intercropping system, respectively. Y_{SW} and Y_{SA} are the yields of wheat and alfalfa in monoculture. The value of LER greater than 1.0 indicates the advantage of intercropping over monoculture, i.e. intercropping systems are more productive than the corresponding monocrops. On the contrary, the value of LER less than 1 indicates that intercrops are disadvantaged in terms of land use.

Actual yield loss (AYL) is the degree of increase or loss in the relative yield of a crop in intercropping system. AYL was determined as follows:

$$\mathrm{AYL}=\mathrm{AYL_W}+\mathrm{AYL_A}$$

$$\mathrm{AYL_W}=\left(\frac{\mathrm{Y_{iW}}/\mathrm{Z_{iW}}}{\mathrm{Y_{sW}}/\mathrm{Z_{sW}}}\right)\text{-}1$$

$$\mathrm{AYL_A}=\left(\frac{\mathrm{Y_{iA}}/\mathrm{Z_{iA}}}{\mathrm{Y_{sA}}/\mathrm{Z_{sA}}}\right)\text{-}1$$

Where AYL_w and AYL_A were the actual yield losses of wheat and alfalfa in intercropping. Z_{iW} and Z_{sw} were the proportion of wheat in intercropping and single cropping, and Z_{iA} and Z_{sA} were the proportion of alfalfa in intercropping and single cropping, respectively. AYL > 0 indicates an increase in intercropping yield, while AYL < 0 indicates a decrease in intercropping yield. The market prices of wheat and alfalfa were used to calculate economic benefits according to the following equations:

Total inputs (SW and SA) = seeds + fertilizers + pesticides + labor costs + machinery costs

Total inputs (W-A) = Total inputs (SW × 2/3) + Total inputs (SA × 1/3)

Net revenue = Total revenue − Total inputs

2.8 Statistical analyses

Statistical analyses were performed using SAS, Version 9.4 (SAS Institute Inc., Cary, NC). The SAS MIXED model procedure was used to evaluate the effects of monoculture and intercropping on root characteristics, soil moisture and yield, yield components and land equivalent ratio, where year was treated as a Repeated Measure variable. The effects of N application, planting pattern and year, and their interactions on yield, yield components and economic profit were also analyzed using the MIXED model procedure. The Tukey's test was performed to evaluate differences between groups and treatments when the model shows significant ($P \leq 0.05$).

3 Results

3.1 Changes in root morphological characteristics in sole and intercropping

Wheat-alfalfa (W-A) intercropping significantly increased wheat root biomass in both growing seasons (Fig. 3). Compared with sole wheat (SW), W-A intercropping increased wheat root biomass in the 0–20 cm soil layer at wheat anthesis by 15.5% and 3.4% under HN and LN, respectively, in the 2022–2023 growing season. In 2023–2024, wheat root biomass in the 0–20 cm soil layer at anthesis was increased by 45.9% and 44.6% under HN and LN, respectively. In the 20–200 cm soil profile, root biomass under W-A at anthesis was increased by 25.6% in 2022–2023 and 26.5% in 2023–2024 under HN. Under LN, root biomass in the 20–200 cm soil layer at anthesis increased by 19.4% and 15.9% in 2022–2023 and 2023–2024, respectively. Wheat root biomass at maturity under intercropping only increased under HN; specifically, in the 0–20 cm soil layer, it increased by 20.6% in 2022–2023 and by 34.8% in 2023–2024. Alfalfa root biomass also increased under the W-A intercropping system (Fig. 3). Compared with SA, alfalfa root biomass in the 0–20 cm soil layer at wheat anthesis was 40.9% and 20.9% higher under HN, and 13.7% and 6.3% higher under LN, in the 2022–2023 and 2023–2024 growing seasons, respectively. Alfalfa root biomass in the 20–200 cm soil layer under W-A intercropping was significantly increased at both wheat anthesis and maturity under both HN and LN conditions.

Compared with SW, root length density of wheat under W-A intercropping at anthesis was increased in both the 0–20 cm and 20–200 cm soil layers under HN and LN in both growing seasons. At maturity, wheat root length density in W-A intercropping only increased under HN, whereas no significant difference was observed between W-A and SW under LN. Compared with SA, root length density of alfalfa in the 0–20 cm soil layer under W-A intercropping was 26.4% and 13.4% higher in 2022–2023, and 28.1% and 29.5% higher in 2023–2024, respectively (Fig. 4).

Compared with SW, wheat root surface area under W–A intercropping in the 0–20 cm soil layer under HN was increased by 12.5% and 18.2% at anthesis, and by 17.7% and 8.0% at maturity in the 2022–2023 and 2023–2024 growing seasons, respectively (Fig. 5). Similarly, in the 20–200 cm soil layer, wheat root surface area under intercropping was increased by 14.4% and 14.4% at anthesis, and by 21.5% and 7.9% at maturity in 2022–2023 and 2023–2024, respectively (Fig. 5). The increase in root surface area was greater for intercropped alfalfa than for wheat. Compared with SA, root surface area of intercropped alfalfa under HN was increased by 21.4% and 23.9% in the 0–20 cm soil layer, and by 36.9% and 16.5% in the 20–200 cm soil layer, at anthesis and maturity in 2022–2023 and 2023–2024, respectively (Fig. 5).

In both growing seasons, intercropping increased root volume in both wheat and alfalfa. Averaged across the 0–200 cm soil profile, root volume of intercropped wheat was increased by 22.2% at anthesis and 23.5% at maturity, while that of intercropped alfalfa was increased by 56.0% and 41.8% at the corresponding stages, respectively. Root volume of both wheat and alfalfa was significantly higher under HN than under LN in both intercropping and monoculture (Fig. 6).

3.2 Soil water content

W–A intercropping affected soil water content before wheat sowing. Under SW, soil water content was stabilized at 20–24% in the 0-200 cm soil profile, whereas under SA, soil water content in the 120–140 cm soil layer was maintained at 10.0% lower than that under SW, resulting in deep soil drying. Before wheat sowing, soil water content in the W-A intercropping system was lower than that under SW but higher than that under SA at depths below 60 cm. At wheat anthesis and maturity, soil water content was higher under W-A intercropping than under SW, especially in the soil profile below 100 cm. Compared with SA, W-A intercropping significantly reduced soil water content under both HN and LN conditions (Fig. 7).

3.3 Wheat yield, yield components, forage yield of alfalfa and water use efficiency

There was no significant difference in wheat spike length between SW and W–A under either HN or LN conditions (Table 1). Except under the LN treatment in 2023–2024, there was no significant difference in spike number between W–A intercropping and SW. Compared with SW, spike number in W-A intercropping under LN increased by 20.6% in 2023–2024. Intercropping increased 1000-grain weight, with an average increase of 9.1% under HN and 13.7% under LN. In 2022–2023, there was no significant difference in grain yield or aboveground biomass between W-A intercropping and SW under HN; however, under LN, grain yield and aboveground biomass in W-A intercropping were lower than those under SW. Compared with the 2022–2023 season, W-A intercropping increased wheat yield by 24.6% and 25.9% under HN and LN, respectively, in 2023–2024. Aboveground biomass also increased by 27.8% and 37.9%, respectively (Table 1). The WUE_B of intercropped wheat increased by 9.6% and 19.8% in the first and second growing seasons, respectively, under HN. Under LN, the WUE_B of intercropped wheat was lower than that of monocropped wheat in the first season but significantly higher in the second season.

Table 1
Grain yield (GY), aboveground biomass (AB), spike length (SL), spike number (SN), kernel number (GN), thousand kernel weight (TKW) and water use efficiency (WUE_B) of wheat under sole and intercropping planting conditions in 2022–2023 and 2023–2024.

Year	Nitrogen application	Plant Pattern	SL	SN	TKW	GY	AB	WUE_B
(Y)	(N)	(P)	(cm)	(m^{-2})	(g)	(kg hm^{-2})	(kg·hm^{-2})	(kg ha^{-1} mm^{-1})
2022–2023	HN	SW-HN	8.3 ± 0.3a	588 ± 78a	44.1 ± 1.7a	7092 ± 192a	17543 ± 1297a	29.6 ± 0.6b
		W/A-HN	8.2 ± 0.2a	516 ± 23b	48.2 ± 0.7a	6185 ± 121a	17052 ± 1124a	32.8 ± 1.1a
	LN	SW-LN	8.4 ± 0.2a	486 ± 41b	44.5 ± 0.7a	6433 ± 163a	15065 ± 1407ab	27.1 ± 1.1b
		W/A-LN	8.2 ± 0.2a	371 ± 18c	50.6 ± 0.2a	3129 ± 145b	11655 ± 121b	24.4 ± 0.8c
2023–2024	HN	SW-HN	11.3 ± 0.6ab	485 ± 29b	47.5 ± 1.8a	6618 ± 189bc	14480 ± 1278b	41.7 ± 2.1b
		W/A-HN	11.4 ± 0.5a	573 ± 35a	51.4 ± 1.9a	8773 ± 229a	20633 ± 2269a	49.3 ± 2.9a
	LN	SW-LN	10.5 ± 0.4b	403 ± 48c	49.4 ± 0.6a	5450 ± 275c	10883 ± 1117c	32.2 ± 1.6b
		W/A-LN	10.8 ± 0.6ab	508 ± 19b	48.6 ± 0.7a	7359 ± 398ab	17533 ± 1189a	45.4 ± 3.1a
P			ns	*	ns	ns	*	ns
N			ns	*	ns	*	*	ns
Y			**	ns	*	*	ns	**
P×N			ns	*	ns	ns	ns	ns
P×Y			**	*	*	**	**	**
N×Y			**	ns	*	*	ns	*
P×N×Y			**	*	*	**	**	**

Different lowercases indicate significant differences among treatments ($p \leq 0.05$) within a single growing season. SW represents sole wheat. W/A represents wheat intercropped with alfalfa. HN represents conventional N application. LN represents reduced N application. *, ** indicates significant at 0.05 and 0.01 level, respectively; ns indicates not significant.

In both growing seasons, W-A intercropping significantly increased alfalfa forage yield. Under HN, W-A intercropping increased alfalfa forage yield by 49.3% in 2022–2023 and 18.7% in 2023–2024. Under LN,

alfalfa forage yield in W-A intercropping was 29.5% and 16.2% higher than that in SA in the two growing seasons, respectively. Compared with SA, the WUE_B of intercropped alfalfa increased by 17.1% and 20.6% in the two growing seasons, respectively (Table 2).

Table 2. Forage yield of different cut, total yield, water use efficiency of alfalfa under sole and intercropping planting conditions in 2022-2023 and 2023-2024.

Year (Y)	Nitrogen application (N)	Plant Pattern (P)	Forage yield (t hm^{-2})			Total yield (t hm^{-2})	WUE_B (kg ha^{-1} mm^{-1})
			1st-cut	2nd-cut	3rd-cut		
2022-2023	HN	SA	5.7±0.2b	6.0±0.4b	7.9±0.7c	19.6±1.3c	27.3±0.9b
		W/A	9.4±0.5a	9.0±0.3a	10.6±0.9a	29.0±1.5a	32.8±1.4a
	LN	SA	6.0±0.3b	5.9±0.6b	6.2±1.1c	18.1±2.1c	23.8±2.9b
		W/A	8.7±0.4a	6.6±0.5ab	9.0±0.3 b	24.3±1.4b	29.4±2.1a
2023-2024	HN	SA	8.8±0.5bc	5.4±0.6a	7.2±0.4b	21.7±1.1b	28.4±1.9b
		W/A	11.6±0.6a	5.9±0.7a	9.2±0.5a	26.7±1.4a	34.5±2.5a
	LN	SA	8.4±1.7c	5.5±0.3a	7.9±0.6b	21.1±0.9b	27.3±1.7b
		W/A	11.2±1.5ab	5.5±0.5a	8.5±0.5ab	25.2±1.2a	33.6±2.3a
P			**	*	*	**	*
N			ns	ns	ns	ns	ns
Y			ns	*	*	ns	*
P×N			**	*	*	**	*
P×Y			*	**	**	**	**
N×Y			ns	*	*	ns	ns
P×N×Y			*	**	**	**	**

Different lowercases indicate significant differences among treatments ($p \leq 0.05$) within a growing season. SA represents sole alfalfa. W/A represents wheat intercropped with alfalfa. HN represents conventional N application. LN represents reduced N application. *, ** indicates significant at 0.05 and 0.01 level, respectively; ns indicates not significant.

3.4 LER, APM, AYL and economic benefit

In both growing seasons, intercropping exhibited land use advantages compared with the sole cropping system, with a land equivalent ratio (LER) of 1.46 and 1.36 under HN, and 1.30 and 1.48 under LN, respectively. Alfalfa had a competitive advantage over wheat during the co-growth period in the first growing season, whereas wheat gained a competitive advantage in the second growing season (Table 3).

Under HN, the actual yield loss of wheat (AYL_W) was 0.12 and that of alfalfa (AYL_A) was 0.49 in the first season; however, in the second season, both wheat and alfalfa in intercropping showed growth advantages compared with their respective sole cropping systems. The total actual yield loss (AYL) of the intercropping system was greater than 0, indicating that W–A intercropping increased yield under HN. Under LN, AYL_W was 0.37 and AYL_A was 0.34, resulting in a total AYL of the intercropping system slightly less than 0, which indicated that wheat–alfalfa intercropping yield decreased under LN in 2022–2023. In 2023–2024, the total AYL of the intercropping system was greater than 0 under both HN and LN (Fig. 8). Under HN, the net revenue of W–A intercropping was 27.5% and 12.1% higher than that of SW, but 10.8% and 12.1% lower than that of SA, in the two growing seasons respectively. Under LN, there was no significant difference in net revenue between W-A intercropping and SW in 2022–2023, but W-A intercropping net revenue was 53.6% higher than that of SW in 2023–2024 (Table 4).

Table 3
Land equivalence ratio and aggressivity of intercropping system of wheat and alfalfa.

Growing season	**Plant pattern**	**Land equivalent ratios (LER)**	**Aggressivity**
2022–2023	W/A-HN	1.46a	0.23b
	W/A-LN	1.30b	0.46a
2023–2024	W/A-HN	1.36a	-0.18b
	W/A-LN	1.48a	-0.41a

Within a growing season, means followed by different lowercase letters are significantly different ($p \leq 0.05$). W/A-HN represents wheat intercropped with alfalfa under conventional N application. W/A-LN represents wheat intercropped with alfalfa under reduced N application

Table 4
The economic benefit of different intercropping and monoculture treatments.

Year	Nitrogen application (N)	Plant Pattern (P)	Production cost (CNY/hm^2)	Total revenue (CNY/hm^2)	Net revenue (CNY/hm^2)
2022-2023	HN	SW	3593	29936 ± 1503c	26342 ± 1503c
		W/A	3421	39758 ± 1392b	36337 ± 1392b
		SA	3079	43823 ± 2526a	40744 ± 2526a
	LN	SW	3172	27975 ± 1582c	24803 ± 1582c
		W/A	3059	30491 ± 722c	27432 ± 722c
		SA	2834	41040 ± 237b	38206 ± 237ab
2023 2024	HN	SW	3583	25417 ± 1112d	21834 ± 1112c
		W/A	3416	43877 ± 2015b	40461 ± 2015b
		SA	3084	49123 ± 1441a	46039 ± 1441a
	LN	SW	3162	19887 ± 1886e	16725 ± 1886d
		W/A	3054	40475 ± 1508c	37421 ± 1508b
		SA	2839	48310 ± 4667a	45471 ± 4667a
	P			**	**
	N			ns	ns
	Y			ns	ns
	P×N			**	**
	P×Y			**	**
	N×Y			ns	ns
	P×N×Y			**	**

Different lowercases indicate significant differences among treatments within a growing season ($p < 0.05$). SA represents sole alfalfa. W/A represents wheat intercropped with alfalfa. HN represents conventional N application. LN represents reduced N application. *, ** indicates significant at 0.05 and 0.01 level, respectively; ns indicates not significant.

4 Discussion

4.1 Intercropping increased root growth and root distribution in wheat and alfalfa crops

The hypothesis that interspecific facilitation between wheat and alfalfa resulted from greater root system growth and proliferation in both crops, is strongly supported by our data. In this study, the RB, RLD, RSA and RV of intercropped wheat in the 0-200 cm soil layer increased by 21.4%, 23.3%, 20.5% and 26.8% respectively, compared with wheat monocropping. Similarly, the RB, RLD, RSA and RV of alfalfa were also higher in the intercropping system than under monocropping. This interspecific facilitation, was giving intercrops a potential advantage over monocrops in capturing and utilizing water and nutrient resources (Latati et al., 2014; Ren et al., 2017; Gong et al., 2020). Alfalfa can fix atmospheric nitrogen biologically, thereby supplying additional nitrogen to the intercropped wheat. The enhanced root growth of wheat may be attributed to its roots expanding toward alfalfa rows to take up nitrogen fixed by alfalfa.Studies on the proso millet-mung bean intercropping showed that the root growth of both crops was promoted to some extent during the cogrowth period because the roots of proso millet expanded laterally toward adjacent rows to obtain the N fixed by the mung bean (Gong et al., 2020). Likewise, when maize was intercropped with soybean, part of the maize root system spread into the soybean row, thereby increasing maize root biomass and root length density (Ren et al., 2017). Another possible explanation is that wheat and alfalfa have relatively independent water and nutrient niches, thereby reducing the intensity of interspecific competition. To acquire more nitrogen from the alfalfa rhizosphere, wheat accelerated root growth by adjusting its root morphological strategies to enhance nitrogen acquisition (Ma et al., 2024). In addition, wheat-alfalfa intercropping may have improved soil porosity, providing conditions for soil loosening and sufficient oxygen and water availability, promoting the growth of wheat and alfalfa root systems. Studies on the soybean-maize intercropping supports this explanation, as the soil physical and chemical characteristics were improved, reshaping the root system architecture, and optimizing root distribution, achieving higher land productivity (Peng et al., 2024).

It should be noted that the increase of root biomass, RLD and RSA in the lower layer of intercropping (20–200 cm) were larger than those in the surface layer (0–20 cm). This suggests that the W-A intercropping has a promoting effect on the growth and development of wheat roots in the subsoil layer. Alfalfa is a deep-rooted crop and the intercropped with alfalfa increases the pores of the subsoil, which is conducive to the growth of wheat roots in deep soil layers. The increase of wheat root biomass and RLD in the subsoil layer could improve water uptake from the subsoil, which has yield advantages (Nakhforoosh et al., 2021; Postma et al., 2014). Deep soil water can be converted to grain more efficiently when rainfall is lower than the water requirement for plant growth during the grain filling period (Lilley and Kirkegaard, 2007).

Root biomass of intercropping under HN conditions was higher than that under other monocropping and N-fertilizer management conditions, indicating that high N and intercropping significantly promoted root growth of wheat and alfalfa. Since N- fertilization affects root morphology and distribution by altering the heterogeneous distribution of N in the soil profile, the N application in this study, increased root-soil contact by increasing RSA, RV, and external junctions of wheat and alfalfa. N application also reduced root diameter and promoted lateral root growth, both of which enhance wheat and alfalfa root topology and ultimately lead to increase in root biomass of wheat and alfalfa (Ramirez-Garcia et al., 2015). Intercropping also increased the root biomass of wheat and alfalfa by increasing the area of contact between wheat and alfalfa roots with the soil; by increasing the number of forks, it increased the fractal dimension and topological index of the root system (Ramirez-Garcia et al., 2015). It is worth noting that in the second

growing season, even under LN condition, the intercropping of wheat and alfalfa had relatively higher root biomass and RLD. The significantly higher increase in wheat root biomass in the second growing season was mainly due to two reasons: on the one hand, the alfalfa root system was more developed in the second year after establishment, and its nitrogen fixation capacity was increased under LN treatment, providing more available nitrogen sources for the growth of wheat root system; on the other hand, wheat was in the adaptation period to the intercropping environment in the first year, and its root plasticity was fully reflected in the second year (Ramirez-Garcia et al., 2015). In addition, the pores of the subsoil were improved by the deep root system of alfalfa, which created favorable soil conditions for the growth of wheat subsoil roots (Peng et al., 2024).

4.2 Intercropping improves water use efficiency in the growing season

During the wheat fallow period (June-September), and compared to the SW, the W-A intercropping reduced soil moisture content in the layer below 60 cm, while compared with SA, W-A intercropping increased the soil moisture content of the soil layer below 60 cm. During the post- harvest (off-season) period, there was no crop growth in the wheat monoculture system. Since soil water was depleted, very little and seasonal precipitation replenishes soil moisture, monocroped wheat plots have higher and balanced soil moisture content before sowing. Compared with SW, W-A intercropping promoted the effective use of soil water storage during the post-wheat harvest season due to the cultivation of alfalfa, thus improving the effectiveness of soil water storage (Ma et al., 2024; Mu et al., 2023). In addition, alfalfa has no idle period and grows vigorously from June to September. In the W-A intercropping, the regrowth of alfalfa significantly reduced topsoil evaporation and enhanced the infiltration capacity of precipitation in July-August. W-A intercropping also reduced the long-term retention of deep soil water and improved the spatio-temporal continuity of water use (Ma et al., 2024; Mu et al., 2023). Compared with SA, W-A intercropping reduces soil water consumption due to the existence of wheat off-season, effectively alleviates the loss of soil water due to the vigorous growth of alfalfa in summer, improves soil water content, and is conducive to improving ecological stability of soil water in farmland (Ali et al., 2021, Ma et al., 2024).

From wheat anthesis to maturity, W-A intercropping reduced soil water depletion and this reduction is important for maintaining soil water ecological stability and improving water use efficiency. Our data showed that the W-A intercropping increased soil water content between anthesis and maturity of wheat, compared to wheat monoculture, but decreased soil water content during the same period compared with alfalfa monoculture. This occurred may be because, the roots of interspecific crops intersected with each other, and the root water absorbing areas were spread out, which expanded the ecological niche for root water uptake, enabling the crops to consume more available water; absorbing and utilizing soil water between the roots of the interspecific crops helped alleviate the impact of water deficit between their roots (Gong et al., 2020). In addition, and because the different water consumption characteristics of the root systems of the two intercropped species, the difference in soil water storage between the crop roots led to the redistribution of soil water transport, thereby reducing the rhizosphere soil water content of the crops that consume less water and increasing the rhizosphere soil water content of the crops that consume more water. This promoted effective utilization of soil water storage, and ultimately improved water use

efficiency (Sun et al., 2018; Mu et al., 2023). In our study the transport and redistribution of soil water may explain the high-water use efficiency of the W-A intercropping system.

4.3 Intercropping system had higher productivity, land use advantages and economic profit

Rational intercropping can improve the land equivalent ratio and increase crop yield per unit land area (Mu et al., 2023; Ma et al., 2024). Our study showed that W-A intercropping did not increase the grain yield of wheat in the first season, but it increased wheat grain yield in the second season. W-A intercropping also improved alfalfa forage yields during both seasons. The no increase in wheat yield in the first season was attributed to the competitive advantage of alfalfa, since its earlier planting allowed alfalfa to occupy the dominant niche, producing a shading effect on wheat (Mu et al., 2023) and to mature early giving an advantage in light competition (Ma et al., 2024). During the cogrowth period, alfalfa roots can extend to deeper soil layers (below 60 cm), occupying a larger area and obtaining water and nutrients from the deep soil layer, which may intensify wheat's competition for nutrients and water (Clement et al., 2022; Sillon et al., 2000; Mu et al., 2023). Studies on spring wheat-alfalfa and maize-alfalfa intercropping have shown that alfalfa has an advantage over wheat and maize in the competition for natural resources (Ma et al., 2023; Mu et al., 2023). The increased wheat grain yield in the second year of the W-A intercropping may resulted from the alfalfa N-fixation that improves soil fertility. The reason for the higher forage yields of intercropped alfalfa is the growth after the wheat harvest. Intercropped alfalfa has a separate growing period (about 120 days) after the wheat harvest. During this period, alfalfa can absorb water and nutrients from the wheat fields. This separate growth phase further results in increased alfalfa biomass production. The yield advantage of the wheat-alfalfa intercropping system is driven by temporal niche differentiation and root vertical stratification plasticity. The growing period of wheat and alfalfa overlaps for about 6 months, and alfalfa can use the light, water and nitrogen resources in the wheat fallow period in the non-overlapping period, while wheat can use the topsoil resources before alfalfa regreening in early spring, which reduces the interspecific competition (Ma et al., 2024). Temporal niche differentiation provides space for root growth: the deep root system of alfalfa expands the deep soil in the non-overlapping period, improves the soil structure, and creates conditions for wheat to absorb deep soil water during the growing period; the topsoil root system of wheat efficiently absorbs nitrogen in the overlapping period, reducing nitrogen competition with alfalfa (Mu et al., 2023).

The forage yield of alfalfa in the two growing seasons and the grain yield of wheat in the second season, improved land use efficiency. In the W-A intercropping, the average land equivalent ratios under HN and LN conditions were 1.41 and 1.35, respectively, indicating that intercropping increased land productivity by 35%-41% compared with SW or SA. Our data support the hypothesis that W-A intercropping can increase productivity and save farmland through interspecific root interactions compared with SW or SA. In addition to yield and land use efficiency, economic benefits are also the main indicators for farmers to choose planting patterns (Raza et al., 2021). A likely reason for the reduced investment in W-A intercropping is that alfalfa strips no longer need to be tilled and seeded, resulting in significant finance savings (Mu et al., 2023). However, the net benefit of intercropping was lower than in SA. Alfalfa is a fed crop with a high protein content and sells for more than wheat. From a net revenue perspective, this is a reasonable choice,

but in practice, SA only produces forage and cannot meet people's needs for food production. Therefore, from that perspective, alfalfa may not be the only option for farmers.

4.4 Perspectives and implications

Our data showed W-A intercropping improved WUE_B and crop yield by enhancing root biomass, RLD, RSA and RV. The increased water uptake and grain yield under W-A intercropping represent a key contribution to improved water use efficiency in semi-arid rain-fed agricultural regions (Ma et al., 2024). In this study, a strip intercropping pattern (e.g., 2–3 rows of wheat paired with 1 row of alfalfa) was used to reduce direct belowground competition between the two species. Alfalfa develops a relatively deep root system, favoring the uptake of water from deep soil layers, whereas wheat forms a shallower adventitious root system, facilitating the use of water in topsoil layers. Timely alfalfa harvest was also critical for optimizing water use: alfalfa was harvested during the critical growth stages of wheat (from jointing to grain filling), allowing priority water use by wheat. After wheat harvest, alfalfa could efficiently utilize rainfall concentrated in July and August. These results indicate that compatible root distribution between intercropped species promotes interspecific facilitation in W-A intercropping systems.

Excessive nitrogen fertilization on the Loess Plateau has caused water eutrophication, soil acidification, and soil degradation (Zhang et al., 2022; Yin et al., 2020). Our study demonstrated that with a 50% reduction in nitrogen fertilizer application, yield losses of intercropped wheat and alfalfa were minimal, while the land equivalent ratio (LER) remained consistently high across both years. Alfalfa exhibits strong biological nitrogen fixation capacity, which can supply nitrogen to associated wheat and reduce reliance on synthetic nitrogen fertilizers (Kermah et al., 2017; Sun et al., 2018).Thus, W-A intercropping under reduced nitrogen input provides a feasible strategy to mitigate environmental problems caused by over-fertilization, while also improving soil fertility and lowering the carbon footprint associated with synthetic fertilizer use. The application of W-A intercropping with reduced nitrogen fertilization shows considerable potential for diversifying agroecosystems and enhancing agricultural sustainability.

Future research should further clarify the regulatory mechanisms underlying root compatibility and interspecific facilitation, optimize management practices including row ratios, harvest schedules, and reduced nitrogen combinations suitable for semi-arid regions, evaluate long-term environmental benefits and sustainability, expand adaptability across different ecological zones, and strengthen integration with modern agricultural technologies. These efforts will help refine the theoretical framework of W-A intercropping, extend its practical application, and support the development of sustainable agriculture.

6 Conclusions

Our study demonstrated that wheat/alfalfa (W/A) intercropping represents a diversified farming system that can enhance crop productivity and land use efficiency on the Loess Plateau and in similar semi-arid ecoregions. W/A intercropping increased root biomass and root length density of both crops under two N fertilization rates via interspecific root complementarity and temporal niche differentiation, thereby improving water use efficiency over the whole growing season. In the intercropping system, alfalfa was dominant during the co-growth period, leading to greater alfalfa forage yield but no significant increase in

wheat grain yield in the first growing season. Wheat yield increased in the subsequent season, benefiting from improved soil structure and enhanced deep soil water utilization mediated by alfalfa roots. Notably, intercropping under reduced N application maintained productivity and profitability comparable to those under conventional N application. Thus, wheat/alfalfa intercropping with reduced N input constitutes a sustainable farming system for the Loess Plateau and similar semi-arid regions (Fig. 9).

Declarations

Conflict of interest

The authors declare no competing interests.

Authors' contributions

Pufang Li and Yongqing Ma contributed to the study conception and design. The collection and analysis of experimental data were completed by Xiaofei Wei and Sha Guo. The chart of experimental results was completed by Jiayuan Jiang, Kexin He, Ting Li, Jiajun Sun and Ciren Dunzhu. The first draft of the manuscript was written by Pufang Li. Baoluo Ma and Jairo A Palta reviews and modifies the initial draft. All authors have read and approved the final manuscript.

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References

1. Ali G, Wang Z, Li X, Jin N, Chu H, Jing L (2021) Deep soil water deficit and recovery in alfalfa fields of the Loess Plateau of China. Field Crop Res 260:107990. https://doi.org/doi.org/10.1016/j.fcr.2020.107990
2. Banik P, Sasmal T, Ghosal PK, Bagchi DK (2000) Evaluation of Mustard (Brassica compestris Var. Toria) and Legume Intercropping under 1:1 and 2:1 Row-Replacement Series Systems. J Agron Crop Sci 185:9–14. https://doi.org/10.1046/j.1439-037X.2000.00388.x
3. Bargaz A, Isaac ME, Jensen ES, Carlsson G (2015) Intercropping of faba bean with wheat under low water availability promotes faba bean nodulation and root growth in deeper soil layers. Procedia Environ Sci 29:111–112. https://doi.org/10.1016/j.proenv.2015.07.188
4. Berti MT, Cecchin A, Samarappuli D, Patel S, Lenssen AW, Moore KJ, Wells SS, Kazula MJ (2021a) Alfalfa established successfully in intercropping with corn in the midwest US. Agronomy 11:1676.

https://doi.org/10.3390/agronomy11081676

5. Berti MT, Lukaschewsky J, Samarappuli DP (2021b) Intercropping alfalfa into silage maize can be more profitable than maize silage followed by spring-seeded alfalfa. Agronomy 11:1196. https://doi.org/10.3390/agronomy11061196
6. Bremner JM (1965) Total nitrogen. In: Norman AG (Ed.), Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties. American Society of Agronomy, Soil Science Society of America, pp. 1149–1178. https://doi.org/10.2134/agronmonogr9.2.c32
7. Clement C, Sleiderink J, Svane SF, Smith AG, Diamantopoulos E, Desbrll DB, Thorup-Kristensen K (2022) Comparing the deep root growth and water uptake of intermediate wheatgrass (Kernza) to alfalfa. Plant Soil 472:369–390. https://doi.org/10.1007/s11104-021-05248-6
8. Gamble J, Baker J, Dalzell B, Wente C, Feyereisen G (2022) Ecohydrology of irrigated silage maize and alfalfa production systems in the upper midwest US. Agr Water Manag:267. https://doi.org/107612.10.2139/ssrn.3993034
9. Gao Y, Duan AW, Qiu XQ, Liu ZG, Sun JS, Zhang JP, Wang HZ (2010) Distribution of roots and root length density in a maize/soybean strip intercropping system. Agr Water Manag 98:199–212. https://doi.org/10.1016/j.agwat.2010.08.021
10. Guo ZY (1992) In: Guo ZY (ed) Shaanxi soil. Part 2. Soil type and character. Science
11. Gong XW, Dang K, Lv S, Zhao G, Tian LX, Luo Y, Feng BL (2020) Interspecific root interactions and water-use efficiency of intercropped proso millet and mung bean. Eur J Agron 115:126034. https://doi.org/10.1016/j.eja.2020.126034
12. Gou F, van Ittersum MK, Wang G, van der Putten PEL, van der Werf W (2016) Yield and yield components of wheat and maize in wheat-maize intercropping in the Netherlands. Eur J Agron 76:17–27. https://doi.org/10.1016/j.eja.2016.01.005
13. Hu F, Gan Y, Chai Q, Feng F, Zhao C, Yu A, Mu Y, Zhang Y (2016) Boosting system productivity through the improved coordination of interspecific competition in maize/pea strip intercropping. Field Crops Res 198:50–60. https://doi.org/10.1016/j.fcr.2016.08.022
14. Jia L, Yu KX, Li ZB, Li P, Zhang JZ, Wang AN, Ma L, Xu GC, Zhang X (2022) Temporal and spatial variation of rainfall erosivity in the Loess Plateau of China and its impact on sediment load. Catean 210:105931. https://doi.org/10.1016/j.catena.2021.105931
15. Jin N, Ren W, Tao B, He L, Ren QF, Li SQ, Yu Q (2018) Effects of water stress on water use efficiency of irrigated and rainfed wheat in the Loess Plateau, China. Sci Total Environ 642:1–11. https://doi.org/10.1016/j.scitotenv.2018.06.028
16. Jungers JM, Yang Y, Fernandez CW, Isbell F, Lehman C, Wyse D, Sheaffer C (2021) Diversifying bioenergy crops increases yield and yield stability by reducing weed abundance. Sci Adv 7:8531. https://doi.org/10.1126/sciadv.abg8531
17. Kanwar RS, Cruse RM, Ghaffarzadeh M, Bakhsh A, Karlen DL, Bailey TB (2005) Corn-soybean and alternative cropping systems effects on NO3-N leaching losses in subsurface drainage water. Appl Eng Agric 21:181–188. http://dx.doi.org/10.13031/2013.18151

18. Kermah M, Franke AC, Adjei-Nsiah S, Ahiabor BDK, Abaidoo RC, Giller KE (2017) Maize-grain legume intercropping for enhanced resource use efficiency and crop productivity in the Guinea savanna of northern Ghana. Field Crop Res 213:38–50. https://doi.org/10.1016/j.fcr.2017.07.008
19. Latati M, Blavet D, Alkama N, Laoufi H, Drevon JJ, Gerard F, Pansu M, Ounane SM (2014) The intercropping cowpea-maize improves soil phosphorus availability and maize yields in an alkaline soil. Plant Soil 385:1–11. https://doi.org/10.1007/s11104-014-2214-6
20. Li L, Li SM, Sun JH, Zhou LL, Bao XG, Zhang HG, Zhang FS (2007) Diversity enhances agricultural productivity via rhizosphere phosphorus facilitation on phosphorus-deficient soils. Proc Nat Acad Sci USA 104:11192–11196. https://doi.org/10.1073/pnas.0704591104
21. Li X, Su D, Yuan Q (2007) Ridge-furrow planting of alfalfa (*Medicago sativa* L.) for improved rainwater harvest in rainfed semiarid areas in Northwest China. Soil Till Res 93:117–125. https://doi.org/10.1016/j.still.2006.03.022
22. Li L, Sun JH, Zhang FS, Guo TW, Bao XG, Smith FA, Smith SE (2006) Root distribution and interactions between intercropped species. Oecologia 147:280–290. https://doi.org/10.1007/s00442-005-0256-4
23. Li PF, Ma BL, Wei XF, Guo S, Ma YQ (2024) Deeper root distribution and optimized root anatomy help improve dryland wheat yield and water use efficiency under low water conditions. Plant Soil 501:437–454. https://doi.org/10.1007/s11104-024-06526-9
24. Lilley JM, Kirkegaard JA (2007) Seasonal variation in the value of subsoil water to wheat: simulation studies in southern New South Wales. Aust J Agr Res 58:1115–1128. https://doi.org/10.1071/ar07046
25. Liu YX, Sun JH, Zhang FF, Li L (2020) The plasticity of root distribution and nitrogen uptake contributes to recovery of maize growth at late growth stages in wheat/maize intercropping. Plant Soil 447:39–53. https://doi.org/10.1007/s11104-019-04034-9
26. Ma BL, Liang BC, Biswas DK, Morrison MJ, McLaughlin NB (2012) The carbon footprint of maize production as affected by nitrogen fertilizer and maize-legume rotations. Nutr Cyc Agroecosys 94:15–31. https://doi.org/10.1007/s10705-012-9522-0
27. Ma HM, Yu XQ, Yu Q, Wu HH, Zhang HL, Pang JY, Gao YZ (2023) Maize/alfalfa intercropping enhances yield and phosphorus acquisition. Field Crop Res 303:109136. https://doi.org/10.1016/j.fcr.2023.109136
28. Ma LS, Li YJ, Wu PT, Zhao XN, Chen XL, Gao XD (2019) Effects of varied water regimes on root development and its relations with soil water under wheat/maize intercropping system. Plant Soil 439:113–130. https://doi.org/10.1007/s11104-018-3800-9
29. Ma QH, Wu YH, Liu YN, Shen YY, Wang ZK (2024) Interspecific interaction and productivity in a dryland wheat/alfalfa strip intercropping. Field Crop Res 309:109335. https://doi.org/10.1016/j.fcr.2024.109335
30. Mahmoud R, Casadebaig P, Hilgert N, Alletto L, Freschet GT, de Mazancourt C, Gaudio N (2022) Species choice and N fertilization influence yield gains through complementarity and selection effects in cereal-legume intercrops. Agron Sustain Dev 42. https://doi.org/10.1007/s13593-022-00754-y
31. Mu L, Su KQ, Zhou T, Yang HM (2023) Yield performance, land and water use, economic profit of irrigated spring wheat/alfalfa intercropping in the inland arid area of northwestern China. Field Crop

Res 303:109116. https://doi.org/10.1016/j.fcr.2023.109116

32. Nakhforoosh A, Nagel KA, Fiorani F, Bodner G (2021) Deep soil exploration vs. topsoil exploitation: distinctive rooting strategies between wheat landraces and wild relatives. Plant Soil 459:397–421. https://doi.org/10.1007/s11104-020-04794-9
33. Peng XY, Ren JB, Chen P, Yang L, Luo K, Yuan XT, Li P, Fu ZD, Li YL, Li YZ, Yang WY, Yong TW (2024) Effects of soil physicochemical environment on the plasticity of root growth and land productivity in maize soybean relay strip intercropping system. Sci Food Agric 104:3865–3882. https://doi.org/10.1002/jsfa.13268
34. Postma JA, Dathe A, Lynch JP (2014) The optimal lateral root branching density for maize depends on nitrogen and phosphorus availability. Plant Physiol 166:590–948. https://doi.org/10.1104/pp.113.233916
35. Ramirez-Garcia J, Martens HJ, Quemada M, Kristian TK (2015) Intercropping effect on root growth and nitrogen uptake at different nitrogen levels. J Plant Ecol 8:380–389. https://doi.org/10.1093/jpe/rtu024
36. Raza MA, Gul H, Wang J (2021) Land productivity and water use efficiency of maize-soybean strip intercropping systems in semi-arid areas: A case study in Punjab Province Pakistan. J Clean Prod 308:127282. https://doi.org/10.1016/j.jclepro.2021.127282
37. Ren YY, Wang XL, Zhang SQ, Palta JA, Chen YL (2017) Influence of spatial arrangement in maize–soybean intercropping on root growth and water use efficiency. Plant Soil 415:131–144. https://doi.org/10.10.1007/s11104-016-3143-3
38. Shen L, Wang XY, Liu TT, Wei WW, Zhang S, Keyhani AB, Li LH, Zhang W (2023) Border row effects on the distribution of root and soil resources in maize–soybean strip intercropping systems. Soil Till Res 233:105812. https://doi.org/10.1016/j.still.2023.105812
39. Sillon JF, Ozier-Lafontaine H, Brisson N (2000) Modelling daily root interactions for water in a tropical shrub and grass alley cropping system. Agrofor Syst 49:131–152. https://doi.org/10.1023/A:1006378708890
40. Simbine MG, Baijukya FP, Onwonga RN (2018) Intermediate maturing soybean produce multiple benefits at 1:2 maize: soybean planting density. J Agr Sci 10:29–46. https://doi.org/10.5539/jas.v10n9p29
41. Stratton AE, Comin JJ, Siddique I, Zak DR, Filipini LD, Lucas RR, Blesh J (2022) Assessing cover crop and intercrop performance along a farm management gradient. Agric Ecosyst Environ 332:107925. https://doi.org/10.1016/j.agee.2022.107925
42. Sun T, Li Z, Wu Q, Sheng T, Du M (2018) Effects of alfalfa intercropping on crop yield, water use efficiency, and overall economic benefit in the Corn Belt of Northeast China. Field Crop Res 216:109–119. https://doi.org/10.1016/j.fcr.2017.11.007
43. Wang ET (2019) Symbiosis Between Rhizobia and Legumes. Ecology and Evolution of Rhizobia. Springer, Singapore. https://doi.org/10.1007/978-981-32-9555-1_1
44. Wang NQ, Wang TQ, Chen Y, Wang M, Lu QF, Wang KG, Dou ZC, Chi ZG, Qiu W, Dai J, Niu L, Cui JY, Wei Z, Zhang FS, Kuemmerli R, Zuo YM (2024) Microbiome convergence enables siderophore-secreting-

rhizobacteria to improve iron nutrition and yield of peanut intercropped with maize. Nat Commun 15:839. https://doi.org/10.1038/s41467-024-45207-0

45. Wilson JB (1988) Shoot competition and root competition. J Appl Ecol 25:279–296. https://doi.org/10.2307/2403626
46. Wu K, Fullen M, An T, Fan Z, Zhou F, Xue G, Wu B (2012) Above–and below–ground interspecific interaction in intercropped maize and potato: a field study using the target tecHNique. Field Crop Res 139:63–70. https://doi.org/10.1016/j.fcr.2012.10.002
47. Wu KX, Jiang CH, Zhou SY, Yang HL (2022) Optimizing arrangement and density in maize and alfalfa intercropping and the reduced incidence of the invasive fall armyworm (Spodoptera frugiperda) in southern China. Field Crops Res 287:108637. https://doi.org/10.1016/j.fcr.2022.108637
48. Xu R, Zhao H, Liu G, You Y, Ma L, Liu N, Zhang Y (2021) Effects of nitrogen and maize plant density on forage yield and nitrogen uptake in an alfalfa-silage maize relay intercropping system in the North China Plain. Field Crop Res 263:108068. https://doi.org/10.1016/j.fcr.2021.108068
49. Yin W, Chai Q, Zhao C, Yu A, Fan Z, Hu F, Fan H, Guo Y, Coulter JA (2020) Water utilization in intercropping: A review. Agric Water Manag 241:106335. https://doi.org/10.1016/j.agwat.2020.106335
50. Yu RP, Lambers H, Callaway RM, Wright AJ, Li L (2021) Belowground facilitation and trait matching: two or three to tango? Trends Plant Sci 26:1227–1235. https://doi.org/10.1016/j.tplants.2021.07.014
51. Zhang DS, Yang LY, Li HB, Tang XY, Hu R, Rengel Z, Zhang FS, Whalley WR, Davies W, Cahill JF, Shen JB (2020) Neighbouring plants modify maize root foraging for phosphorus: coupling nutrients and neighbours for improved nutrient-use efficiency. New Phytol 226:244–253. https://doi.org/10.1111/nph.16206
52. Zhang F, Li L (2003) Using competitive and facilitative interactions in intercropping ystems enhances crop productivity and nutrient-use efficiency. Plant Soil 248:305–312. https://doi.org/10.1023/A:1022352229863
53. Zhang G, Yang Z, Dong S (2011) Interspecific competitiveness affects the total biomass yield in an alfalfa and corn intercropping system. Field Crops Res 124:66–73. https://doi.org/10.1016/j.fcr.2011.06.006
54. Zhang Y, Sun Z, Su Z, Du G, Bai W, Wang Q, Wang R, Nie J, Sun T, Feng C, Zhang Z, Yang N, Zhang X, Evers JB, van der Werf W, Zhang L (2022) Root plasticity and interspecific complementarity improve yields and water use efficiency of maize/soy bean intercropping in a water-limited condition. Field Crop Res 282:108523. https://doi.org/10.1016/j.fcr.2022.108523

Figures

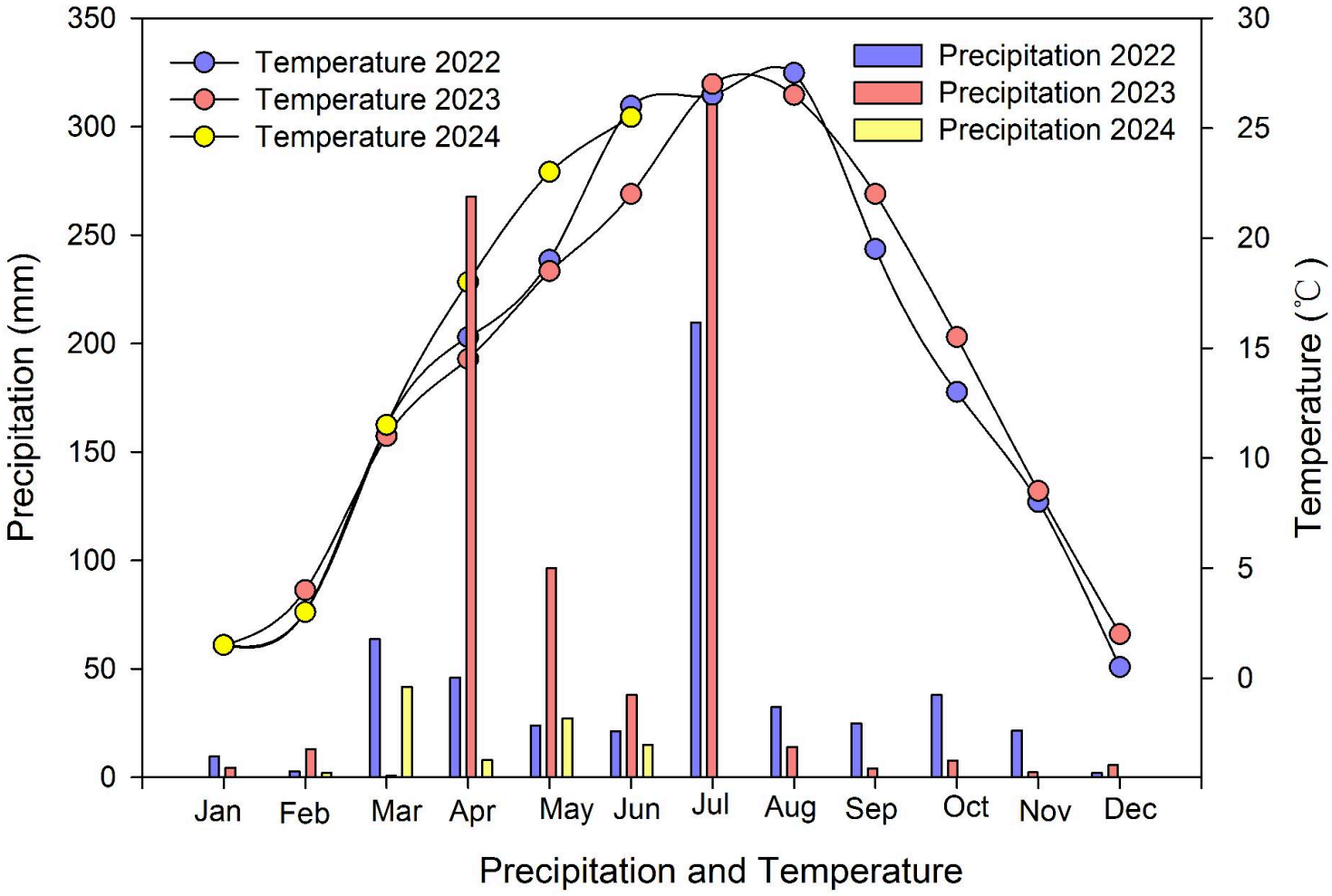


Figure 1

Precipitation and temperature during alfalfa and winter wheat growth seasons of 2022-2023, and 2023-2024.

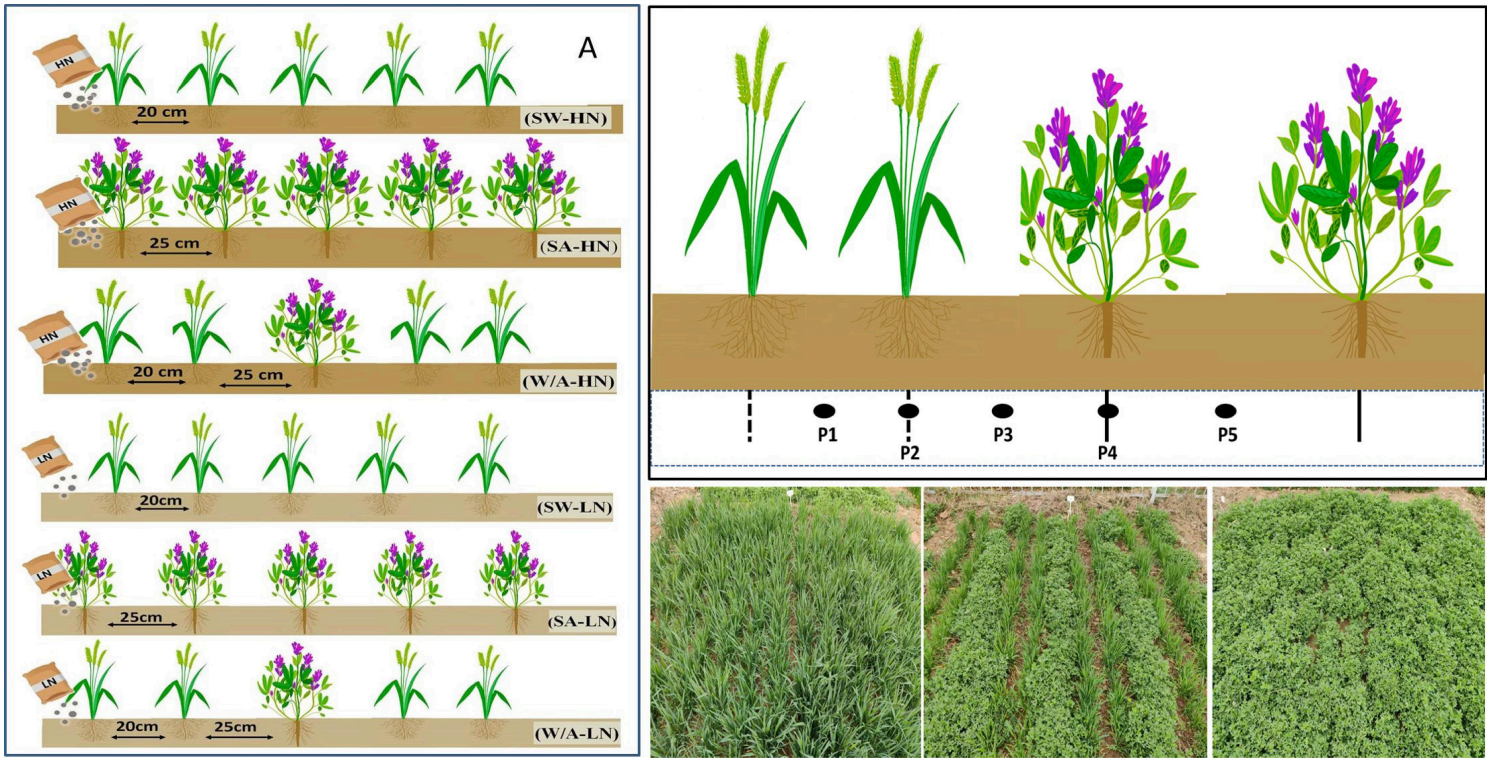


Figure 2

The schematic representation of the sole cropping and intercropping system (A) and soil and root samples: (P1) at the inter-row of wheat, (P2) intra-row of wheat, (P3) adjacent interrow of wheat and alfalfa, (P4) intra-row of alfalfa and (P5) inter-row of alfalfa.

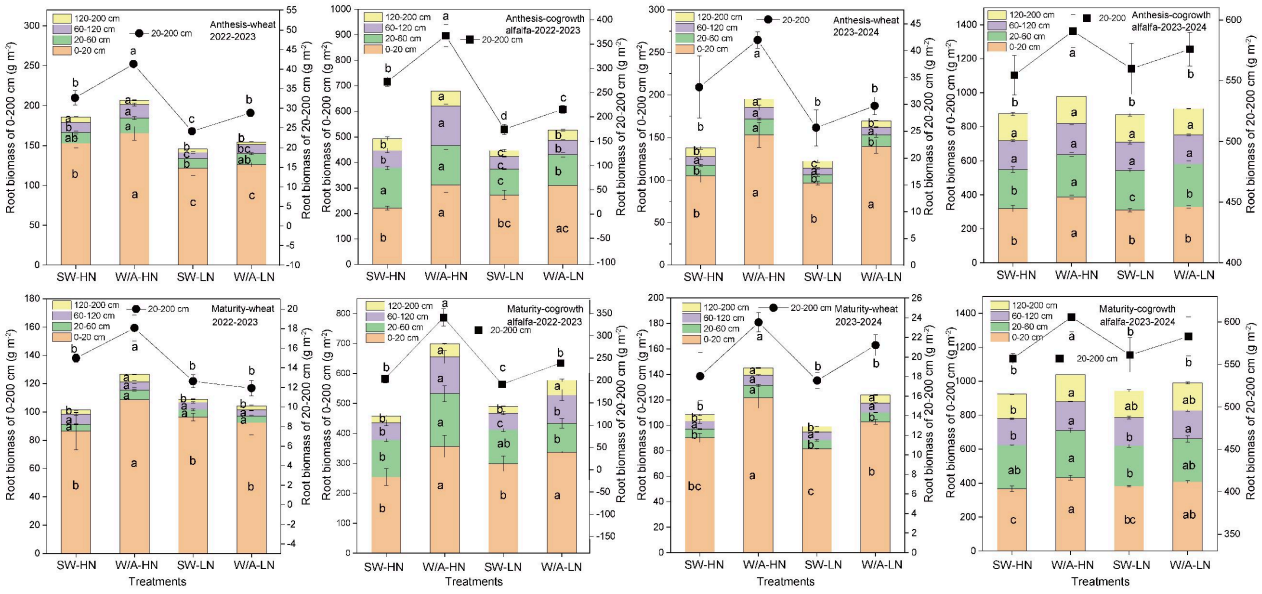


Figure 3

Root biomass in sole cropping and intercropping system under high and low nitrogen application conditions. Different lowercase letters indicate significant differences among treatments ($P \leq 0.05$). SW-HN represents sole wheat under conventional N application. SW-LN represents sole wheat under reduced N application. W/A-HN represents wheat intercropped with alfalfa under conventional N application. W/A-LN represents wheat intercropped with alfalfa under reduced N application. SA-HN represents sole alfalfa under conventional N application. SA-LN represents sole alfalfa under reduced N application.

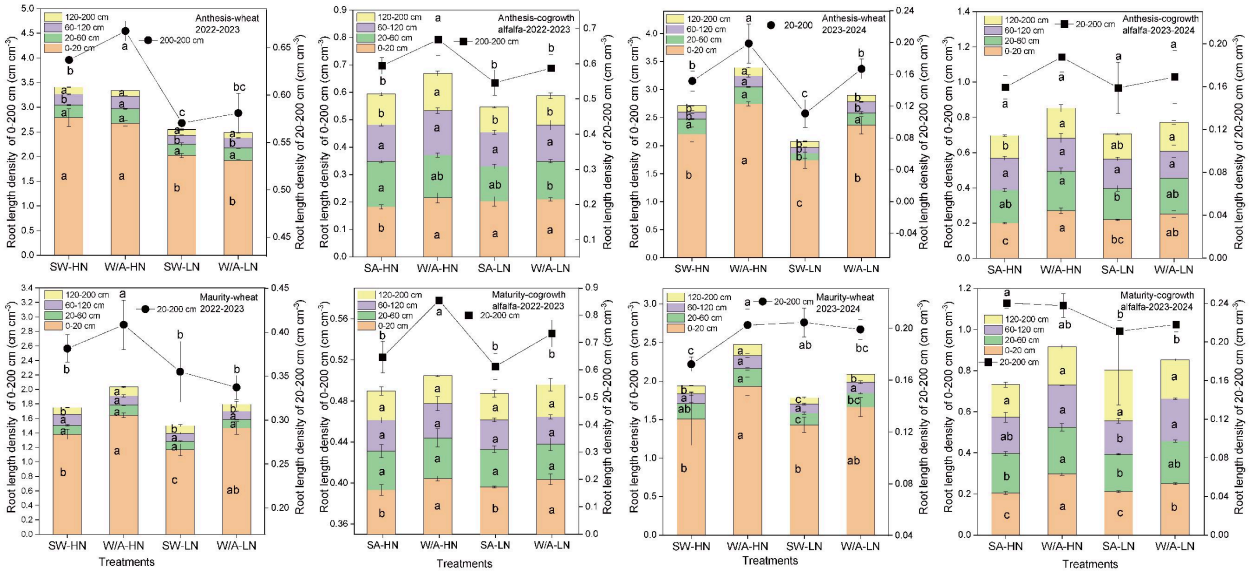


Figure 4

Root length density in sole cropping and intercropping system under high and low nitrogen application conditions. Different lowercase letters indicate significant differences among treatments ($P \leq 0.05$). SW-HN represents sole wheat under conventional N application. SW-LN represents sole wheat under reduced N application. W/A-HN represents wheat intercropped with alfalfa under conventional N application. W/A-LN represents wheat intercropped with alfalfa under reduced N application. SA-HN represents sole alfalfa under conventional N application. SA-LN represents sole alfalfa under reduced N application.

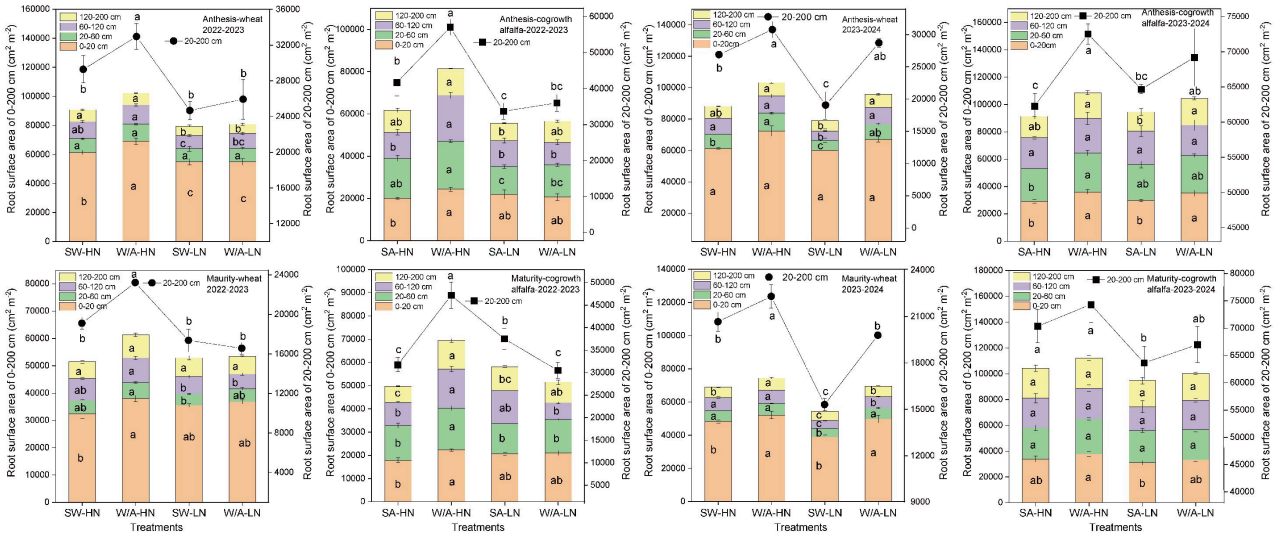


Figure 5

Root specific area in sole cropping and intercropping system under high and low nitrogen application conditions. Different lowercase letters indicate significant differences among treatments ($P \leq 0.05$). SW-HN represents sole wheat under conventional N application. SW-LN represents sole wheat under reduced N application. W/A-HN represents wheat intercropped with alfalfa under conventional N application. W/A-LN represents wheat intercropped with alfalfa under reduced N application. SA-HN represents sole alfalfa under conventional N application. SA-LN represents sole alfalfa under reduced N application.

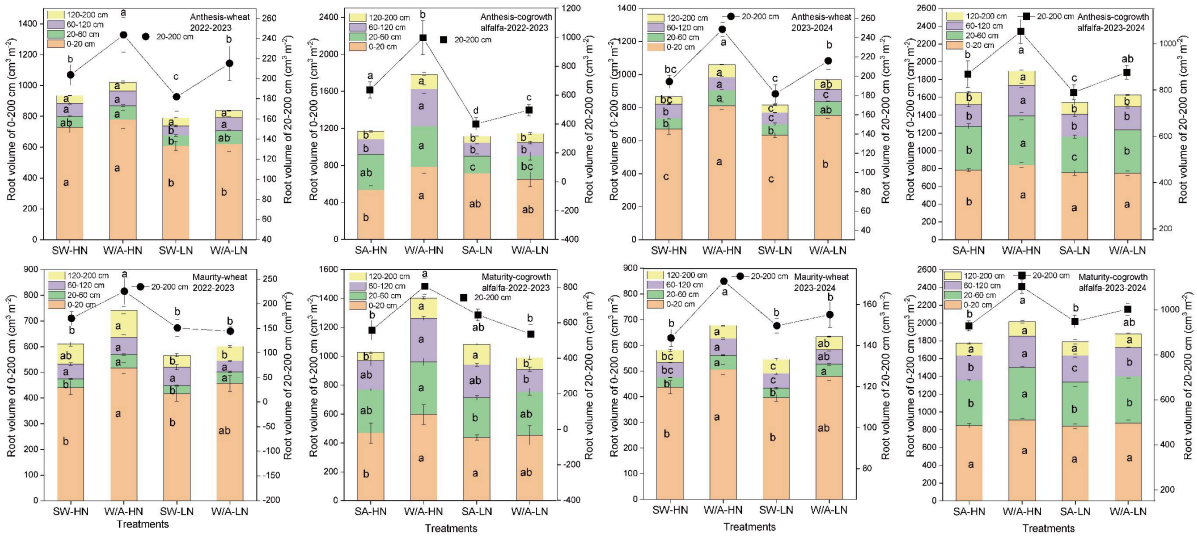


Figure 6

Root volume in sole cropping and intercropping system under high and low nitrogen application conditions. Different lowercase letters indicate significant differences among treatments (P≤ 0.05). SW-HN represents sole wheat under conventional N application. SW-LN represents sole wheat under reduced N application. W/A-HN represents wheat intercropped with alfalfa under conventional N application. W/A-LN represents wheat intercropped with alfalfa under reduced N application. SA-HN represents sole alfalfa under conventional N application. SA-LN represents sole alfalfa under reduced N application.

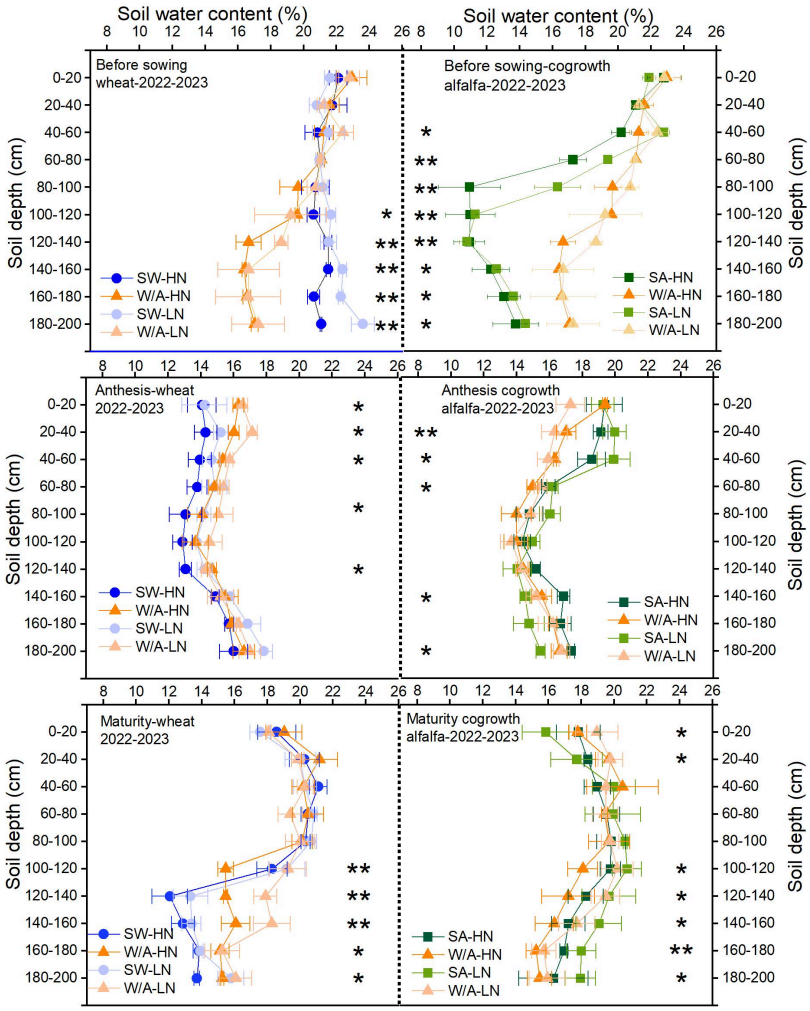


Figure 7

Soil water content in sole cropping and intercropping system under high and low nitrogen application conditions. SW-HN represents sole wheat under conventional N application. SW-LN represents sole wheat under reduced N application. W/A-HN represents wheat intercropped with alfalfa under conventional N application. W/A-LN represents wheat intercropped with alfalfa under reduced N application. SA-HN represents sole alfalfa under conventional N application. SA-LN represents sole alfalfa under reduced N application.

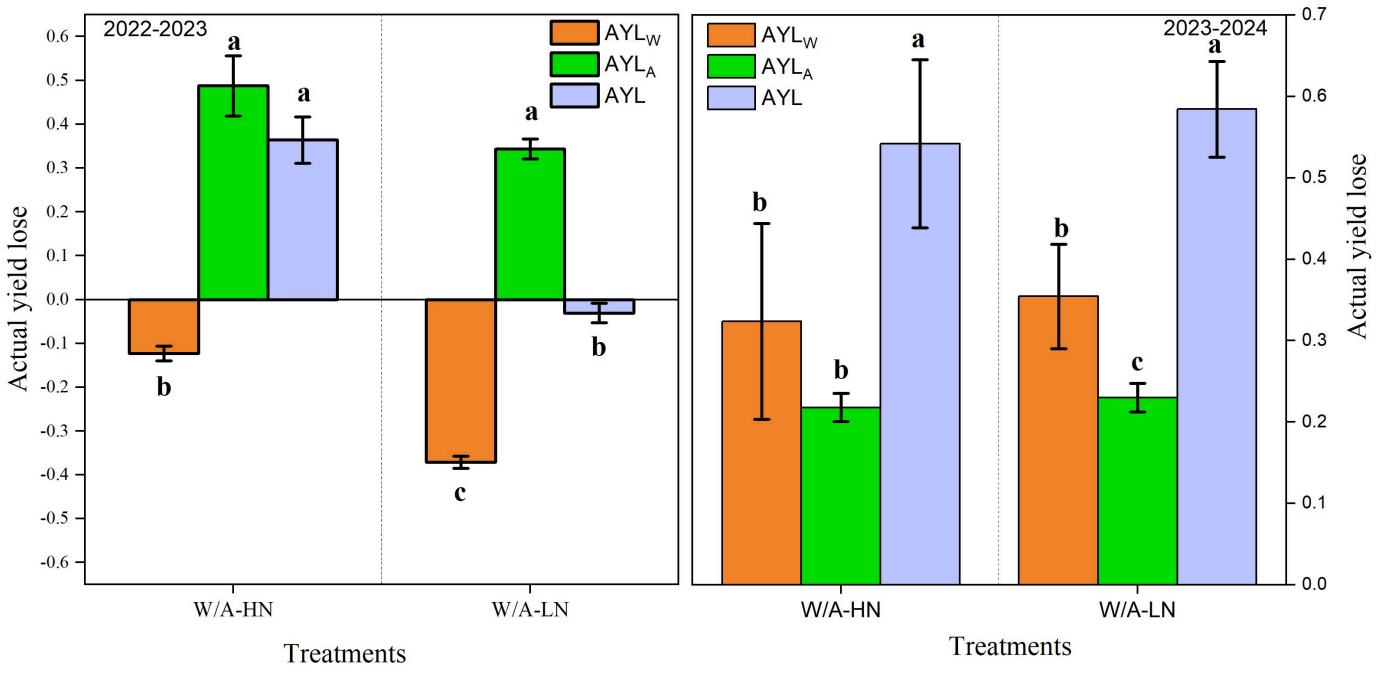


Figure 8

Actual losses in wheat/alfalfa intercropping. AYL_W represents yield loss of wheat. AYL_A represents yield loss of alfalfa. AYL represents total yield loss.

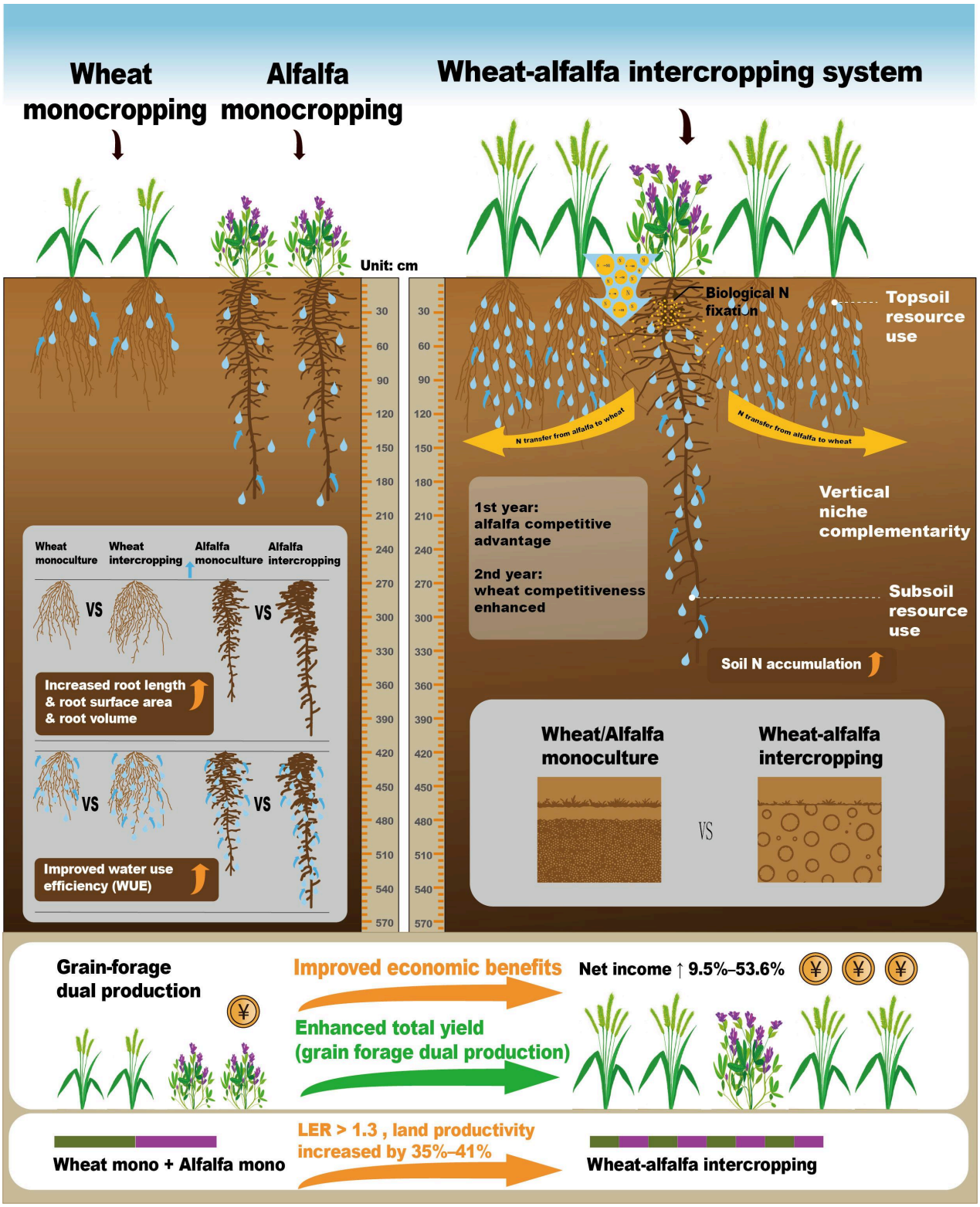


Figure 9

Schematic diagram of sole cropping and wheat-alfalfa intercropping systems. Intercropping effectively improves root morphology and water use efficiency of both crops. Alfalfa fixes and transfers nitrogen to wheat, and root vertical stratification enhances soil resource utilization and nitrogen accumulation. Alfalfa dominates competition in the first growing season, while wheat prevails in the second season. The intercropping system realizes grain-forage co-production and yield improvement. It exhibits superior land-

use efficiency with LER > 1.3, 35%–41% higher land productivity, increased total biomass, and 9.5%–53.6% higher net income, demonstrating obvious economic and ecological benefits.