WATER–NITROGEN COUPLING EFFECTS ON GROWTH DYNAMICS AND ALKALOID ACCUMULATION IN DRIP-IRRIGATED *SOPHORA ALOPECUROIDES* L.

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Abstract. To increase yield and alkaloid accumulation in *Sophora alopecuroides* L. through optimized water–nitrogen management, this study was conducted to investigate the effects of different irrigation and nitrogen regimes via a split-plot design with two irrigation levels (W1: deficit irrigation at 1080 m-3 ha^{-1}, 40% reduction; W2: conventional irrigation at 1800 m^{-3} ha^{-1}) and three nitrogen application rates (N1: 0 kg ha^{-1}; N2: 105 kg ha^{-1}; and N3: 210 kg ha^{-1}). Dynamic growth parameters (plant height, stem diameter, and dry matter accumulation), soil properties (0–60 cm depth), and leaf alkaloid content and accumulation were analyzed. The results demonstrated that the W2N2 regime maximized the yield (3612.67 kg ha^{-1}) and partial factor productivity of nitrogen (NPP: 36.62 kg kg^{-1}) while reducing the topsoil pH, whereas the W1N3 regime resulted in the greatest alkaloid accumulation (16.15 mg $plant^{-1}$) and water use efficiency (IWP: 2.86 kg m^{-3}) across all growth stages. The yield was significantly correlated with growth metrics (plant height: $P < 0.001$; stem diameter: $P < 0.01$) but not with alkaloids ($P > 0.05$). The W1N3 regime (deficit irrigation + high nitrogen) is recommended for optimizing alkaloid production in *S. alopecuroides* cultivation.
Keywords: *alkaloid content, dry matter, yield, soil environment*

Introduction

Sophora alopecuroides L. (Fabaceae), a perennial herb indigenous to the arid and semiarid steppes and desert regions of Northwest China, is characterized by high ecological and medicinal value (Wang et al., 2020). This species is enriched in alkaloids throughout its tissues, with contents ranging from 6.11–8.03% in its aerial parts; thus, it is positioned as a primary botanical source for plant-derived pesticides. The increasing global demand for green agricultural products has increased the use of S. alopecuroides as a globally significant medicinal resource because of its natural bioactive constituents and eco-compatibility (Zhang et al., 2023). Nevertheless, current supply chains remain heavily dependent on wild populations, and the implementation of unsustainable harvesting practices has triggered population decline in multiple natural stands, posing severe threats to biodiversity and ecosystem stability (Guo et al., 2024). Consequently, developing high-efficiency cultivation methods has become imperative for sustainable resource use.

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Alkaloids, which are the core secondary metabolites in *S. alopecuroides*, exhibit content variations governed not only by genetic factors but also by environmental conditions and cultivation practices. Water availability, a critical growth determinant, notably regulates plant morphogenesis and metabolic processes (Yang et al., 2018). Empirical evidence has confirmed that moderate drought stress results in the activation of secondary metabolic pathways, increasing alkaloid biosynthesis (Zuo et al., 2022), whereas excessive irrigation suppresses alkaloid synthesis in S. alopecuroides, leading to a significant reduction in the alkaloid content (Gao et al., 2008). Furthermore, nitrogen—an essential macronutrient and structural component of alkaloids—plays pivotal roles in shaping plant metabolism. Controlled nitrogen application notably increases plant architecture indices and alkaloid contents (Ji et al., 2008). Consequently, optimized water–nitrogen management represents the key pathway for synergistically increasing the yield of and alkaloid accumulation in *S. alopecuroides*.

Chinese agriculture faces the dual challenges of water scarcity and low fertilizer use efficiency, yet excessive irrigation and nitrogen application remain pervasive, resulting in nitrogen leaching and soil degradation issues. Integrated water–fertilizer technology effectively enhances nutrient–water use efficiency, conserves resources, enables a balanced nutrient supply, mitigates environmental impacts, and improves soil health (Wang et al., 2025). Previous studies on other medicinal plants have demonstrated that water–nitrogen coupling significantly promotes root growth and glycyrrhizin synthesis in *Glycyrrhiza uralensis* (Liu et al., 2025), optimizes dry matter partitioning in *Astragalus membranaceus* (Wei et al., 2021), and regulates tanshinone biosynthesis in *Salvia miltiorrhiza* (Liu, 2021). Nevertheless, critical knowledge gaps persist regarding the growth dynamics and alkaloid accumulation patterns of Sophora alopecuroides under different water–nitrogen regimes. Consequently, determining whether drip irrigation–fertigation integration can facilitate the optimization of the water–nitrogen supply to increase alkaloid accumulation constitutes an urgent research priority.

Therefore, in this study, different water–nitrogen regimes were used to investigate the response patterns in terms of the growth parameters, dry matter accumulation, and alkaloid dynamics of *S. alopecuroides*, with the goal of identifying the optimal coupling strategy for maximizing alkaloid production and ultimately providing evidence-based water–fertilizer management strategies for its cultivation.

Materials and methods

Experimental site

A field experiment was conducted at the Bozhou Water Conservancy and Irrigation Station in 2024 (*Fig. 1*). The experimental site is located in Tuoli town, Jinghe County (82°28′E, 44°61′N), which is characterized by a northern temperate arid desert continental climate. The region is characterized by low precipitation, high evaporation rates, hot and dry summers, frequent strong winds in spring and summer, long and severe winters, abundant sunshine, ample thermal resources, significant diurnal temperature variation, and a long frost-free period. The annual average precipitation is 114.95 mm, the annual average temperature is 8.65°C, the annual average sunshine duration is 2530.6 h, and the annual average frost-free period is 175 d (Zhang et al., 2024). The temperature and precipitation during the entire growth period of *Sophora alopecuroides* in 2024 are shown in *Figure 2*. The soil in the experimental area is classified as medium loam, and the physicochemical properties of the 0–60 cm soil layer are presented in *Table 1*.

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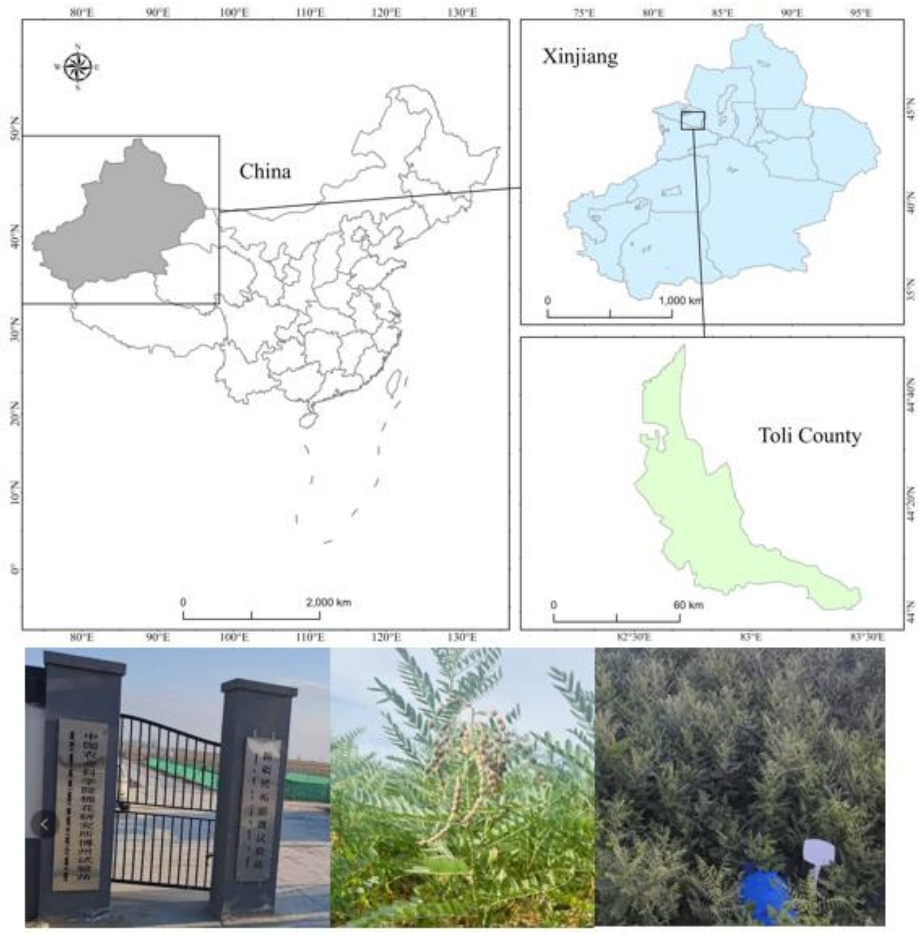

Figure 1. *Location of the experimental site*

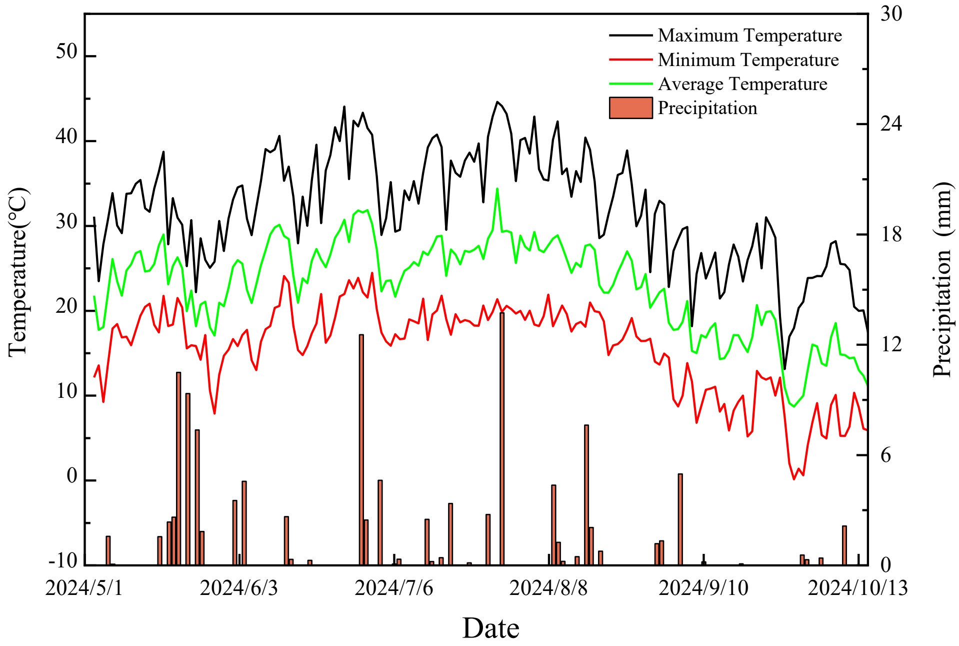

Figure 2. *Daily maximum temperature, minimum temperature, average temperature, and precipitation during the growing season of Sophora alopecuroides in 2024*

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Table 1. *Soil physicochemical properties of the experimental area in the 0–60 cm layer*

Soil depth (cm)	pH	EC (μs·m-^{1})	Nitrate nitrogen (mg kg^{-1})	Available phosphorus (mg kg^{-1})	Available potassium (mg kg^{-1})	Total nitrogen (mg kg^{-1})	Organic matter (g kg^{-1})
0-20	8.35	444.02	6.11	27.24	218.04	713.54	5.26
20-40	8.17	483.87	2.86	17.34	165.31	613.46	4.34
20-40	8.36	346.06	1.39	5.76	71.49	273.24	2.42

Experimental site

A split-plot design was employed in this experiment, with the irrigation amount employed as the main plot variable and the nitrogen application rate employed as the subplot variable. In this experiment, based on the local standard for artificial cultivation of Sophora alopecuroides in Gansu Province (DB62/T 4413–2021), two irrigation levels were established: W1 (reduced irrigation by 40%, 1080 m^{-3} ha^{-1}) and W2 (conventional irrigation, 1800 m^{-3} ha^{-1}). Three nitrogen application levels were designed, namely N1 (no nitrogen applied), N2 (105 kg ha^{-1}), and N3 (210 kg ha^{-1}). A total of six treatment combinations were generated. Urea (containing 46% pure N) was employed as the nitrogen fertilizer, and potassium dihydrogen phosphate (containing 52% P_2O_5 and 34% K_2O) was selected as the phosphorus and potassium fertilizer, with an application rate of 210 kg hm^{-2}. Each treatment involved the use of 5 irrigations, along with two topdressings of nitrogen fertilizer and potassium dihydrogen phosphate. Irrigation was initiated in mid-to-late May and concluded in mid-to-late August, with an interval of 20-25 days between irrigations. Nitrogen fertilizer and potassium dihydrogen phosphate were applied as topdressing in mid-May, accounting for 2/3 of the total amount. The remainder (1/3) was applied in early July. For each treatment, phosphorus, potassium, and nitrogen fertilizers were applied simultaneously through drip irrigation. The nitrogen application schedule for each irrigation is detailed in *Table 2*.

Each treatment was replicated 3 times, totaling 18 plots, with each plot measuring 31.38 m² in size (13.6 m × 2.3 m). Sophora alopecuroides was planted under plastic film mulching, with a pattern of four rows per film. The row spacing was 30 cm, the plant spacing was 15 cm, the drip tape spacing was 50 cm, and the distance from the drip tape to the plants was 5 cm (*Fig. 3*). The drip irrigation system and accompanying fertigation equipment were provided by the Bozhou Hydraulic Irrigation Experimental Station. The drip tape was operated at a working pressure of 0.1 MPa, with a designed flow rate of 3.2 L/h per emitter. A Venturi injector was used for fertigation, and the system pressure was maintained stable during fertilization to ensure uniform injection of the fertilizer solution. During the initial occurrence of aphids (early June, when the leaf curling rate reached approximately 5%), a single uniform spray of 10% imidacloprid wettable powder at a dilution of 2500:1 was applied. For powdery mildew, a single uniform spray of 62.25% mancozeb · myclobutanil wettable powder at a dilution of 2000:1 was applied at the early stage of the disease (late June, when sporadic powdery spots appeared on the lower leaves). Pesticide applications were conducted under clear weather conditions with wind speeds below level 3. Manual weeding was performed within 2–3 days after irrigation, when the soil was soft, for a total of 6 times throughout the entire growth period to ensure that no weed competition occurred within the plots.

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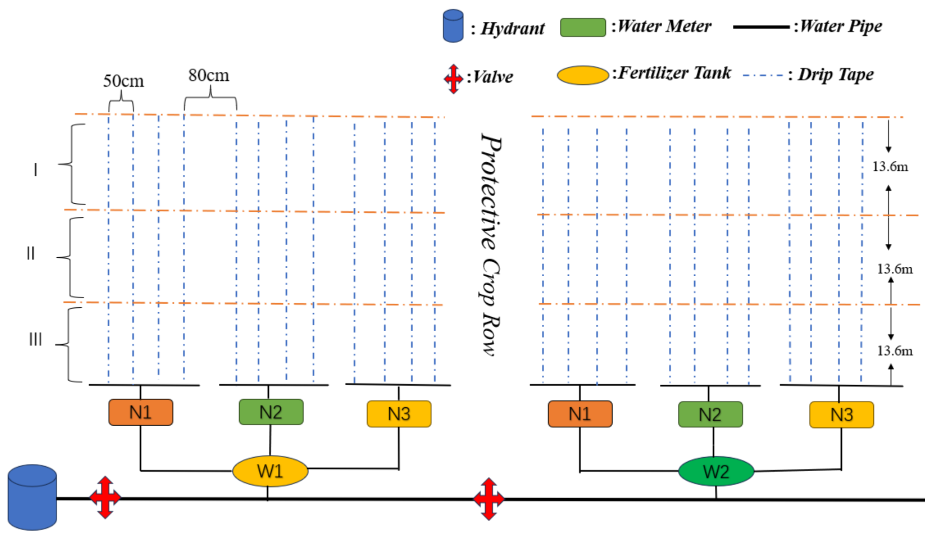

Figure 3. *Schematic diagram of the Sophora alopecuroides planting pattern*

Table 2. *Irrigation dates and fertilization ratios for the different treatments*

Treatment	Date					Nitrogen application rate (kg hm⁻²)	Nitrogen application rate (kg hm⁻²)
	5.15	6.7	7.4	7.29	8.23		
W1N1	0	0	0	0	0	0	1080
W1N2	70	0	35	0	0	105	1080
W1N3	140	0	70	0	0	210	1080
W2N1	0	0	0	0	0	0	1800
W2N2	70	0	35	0	0	105	1800
W2N3	140	0	70	0	0	210	1800

Measurement items and methods

Plant height and stem diameter

Measurements commenced 10 days after the initiation of the water treatments, i.e., on May 26, 2024, and they concluded in September of the same year. In each treatment plot, 10 representative plants with uniform growth were selected as fixed samples and measured at five time points: May 26, June 20, July 15, August 9, and September 4 (approximately 25 ± 3 days apart). The plant height was measured from the soil surface at the stem base to the apical growing point via a steel tape measure with an accuracy of 0.1 cm. The stem diameter was measured 2 cm above the stem base with a digital Vernier caliper with an accuracy of 0.01 mm while avoiding nodes and deformities. Two perpendicular measurements were obtained and averaged.

Dry matter accumulation

Samples were collected at four key growth stages: the vegetative stage (late May, May 26), the flowering stage (mid-July, July 15), the fruiting stage (mid-to-late August, August 20), and the harvest stage (early September, September 4). In each plot, 10 representative plants were cut at ground level. The aboveground parts were immediately

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separated into stems and leaves, placed in prelabeled parchment paper bags, and dried in a forced-air oven at 105°C for 30 min, followed by drying at a temperature of 75°C for 48–72 h until a constant weight was reached. The dry weights of the stems and leaves were measured via an electronic balance with an accuracy of 0.001 g, and the dry matter allocation ratio of each organ per plant was calculated.

Soil environmental parameter determination

Soil samples were collected after the final fertilization using a five-point sampling method from each water-nitrogen coupling treatment plot. Samples were taken from three soil depths (0–20, 20–40, and 40–60 cm), with three replicates per depth. Soil pH and electrical conductivity were measured with a Mettler Toledo FE38 pH meter. The soil moisture content was determined by oven-drying at 105 °C to a constant weight. Soil nitrate-N was analyzed using a UV-2600 ultraviolet–visible spectrophotometer, and soil total N was determined with a KDN-812 Kjeldahl nitrogen analyzer (Ma, 2024). All the instruments used were supplied by Nanjing Kexiang Instrument Equipment Co., Ltd.

Yield and water–nitrogen use efficiency

The plants in each plot were harvested separately, the collected plants were weighed with an electronic scale, and the results were converted into the yield per hectare. The irrigation water productivity (IWP) and partial factor productivity of nitrogen (NPP) were calculated as follows (Wang et al., 2024):

$$IWP = Y / I \tag{Eq.1}$$

where IWP is the water use efficiency of S. alopecuroides (kg·m^{-3}), Y is the yield (kg·hm^{-2}), and I is the total irrigation amount during the growth period (hm^{-2} m^{-3}).

$$NPP = Y / N \tag{Eq.2}$$

where PN is the nitrogen partial productivity (kg kg^{-1}), Y is the yield (kg hm^{-2}), and N is the amount of applied pure nitrogen (kg hm^{-2}).

Alkaloid content determination

Measurements were conducted 10 days after the water treatment from May to September 2024 at 25-day intervals (May 26, June 20, July 15, August 9, and September 4), totaling five measurements. In each plot, 15 representative plants were selected, dried at 105°C for 30 min to deactivate enzymes, further dried at 75°C, ground with a pulverizer, sieved through a 60-mesh sieve, and stored in sealed plastic bags. The total alkaloid content in the whole plant was determined via ultraviolet spectrophotometry (Huang et al., 2024).

Data processing

The experimental data were collated using Excel 2019. Data analysis and graphing were performed using SPSS 17 and Origin 2021 software, respectively. Analysis of variance (ANOVA) and the least significant difference (LSD) test were applied for variance analysis and multiple comparisons of the data, respectively.

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Results and analysis

Effects of water–nitrogen coupling on the soil moisture content

As shown in *Figure 4* and *Table 3*, the irrigation amount and nitrogen application rate significantly influenced the soil moisture content in the 0–20 cm and 20–40 cm soil layers, whereas the interaction between water and nitrogen significantly affected only the soil moisture content in the 20–40 cm soil layer ($P < 0.05$). In the 0–20 cm soil layer, the soil moisture content ranged from 8.75% to 14.53%. In the 20–40 cm soil layer, the soil moisture content ranged from 8.8% to 14.5% and increased with increasing irrigation amount. Compared with that in the N1 treatment, the soil water content in the N3 treatment significantly increased by 27.6% ($P < 0.05$). However, under the W2 regime, there was no significant difference in the soil moisture content among all the treatments.

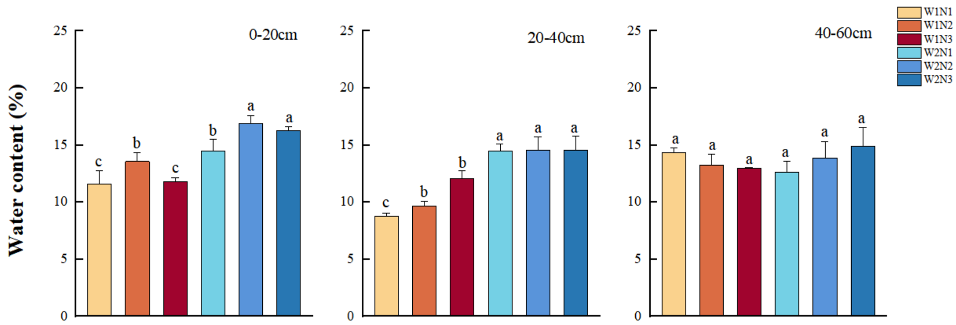

Figure 4. *Soil moisture content in the different soil layers under the different water–nitrogen coupling treatments. The different letters in each column indicate that the difference between the different nitrogen treatments under the same irrigation treatment conditions was significant (P < 0.05). The error bars represent ± SDs*

Table 3. *Variance analysis of the effects of water-–nitrogen coupling on soil moisture content in different soil layers*

ANOVA			
Soil depth (cm)	**0-20**	**20-40**	**40-60**
W	***	***	ns
N	**	**	ns
W*N	ns	**	ns

ns denotes not significant; * denotes a significant correlation at the P < 0.05 level; ** denotes a significant correlation at the P < 0.01 level; and *** denotes a significant correlation at the P < 0.001 level

Effects of water–nitrogen coupling on soil nitrate and total nitrogen contents.

As shown in *Figure 5* and *Table 4*, irrigation, nitrogen application, and their interaction had significant effects on the contents of soil total nitrogen and nitrate nitrogen in the 0–60 cm soil layer ($P < 0.05$). In the 0–20 cm layer, the soil total nitrogen content ranged from 0.19 to 0.80 g kg^{-1}. In the W1 treatment, compared with the N3 treatment, the N2 treatment significantly increased total nitrogen by 5.7% ($P < 0.05$), but the difference was not significant. Compared with the N1 treatment, the N2 and N3 treatments significantly

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increased total nitrogen by 24.4% and 11.1%, respectively ($P < 0.05$). The soil nitrate nitrogen content ranged from 0.22 to 8.17 mg kg^{-1}. Compared with N1, N2 and N3 significantly increased nitrate nitrogen by 69.3% and 39.9%, respectively. Compared with N1, N2 and N3 also significantly increased nitrate nitrogen by 42.4% and 43.8%, respectively ($P < 0.05$).

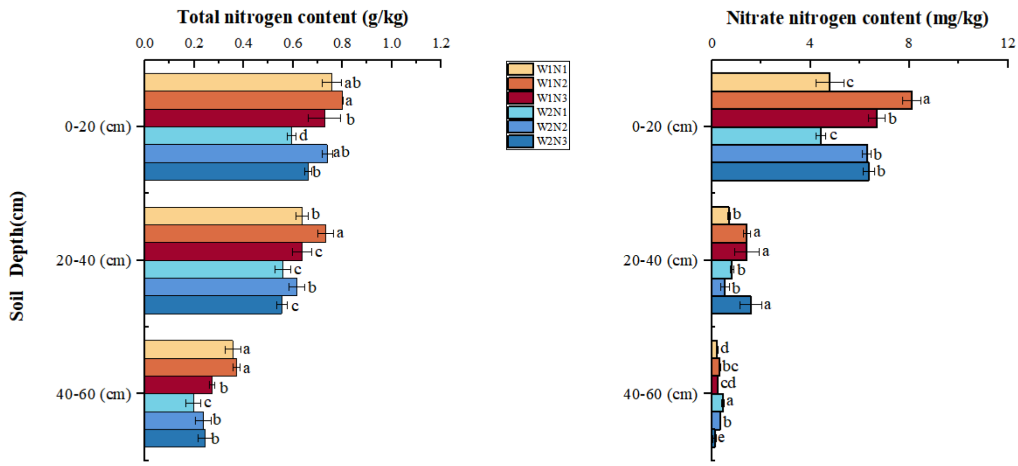

Figure 5. *Soil nitrate nitrogen and total nitrogen contents in different soil layers under combined water and nitrogen treatments. The different letters in each column indicate that the difference between the different nitrogen treatments under the same irrigation treatment conditions was significant (P < 0.05). The error bars represent ± SDs*

Table 4. *Analysis of variance the effects of water and nitrogen coupling on soil total nitrogen and nitrate nitrogen in different soil layers*

ANOVA						
	Nitrate			Total nitrogen		
Soil depth (cm)	**0-20**	**20-40**	**40-60**	**0-20**	**20-40**	**40-60**
W	***	***	ns	***	***	***
N	**	**	ns	**	***	*
W*N	ns	**	ns	*	***	***

ns denotes not significant; * denotes a significant correlation at the P < 0.05 level; ** denotes a significant correlation at the P < 0.01 level; and *** denotes a significant correlation at the P < 0.001 level

Effects of water–nitrogen coupling on the pH and electrical conductivity

As shown in *Figure 6* and *Table 5*, the soil pH in the 0–20 cm layer ranged from 7.58 to 8.75. The amount of irrigation, nitrogen application rate, and their interaction significantly influenced the soil pH ($P < 0.05$). The pH value in each treatment decreased with increasing irrigation amount. Under the W2 treatment condition, the pH values in the N2 and N3 treatments significantly decreased by 11.9% and 12.8% ($P < 0.05$), respectively, compared with those in the N1 treatment, whereas under the W1 treatment condition, no significant differences in pH were observed among all the treatments. The amount of irrigation significantly influenced the pH value in the 20–40 cm and 40–60 cm soil layers ($P < 0.05$), whereas the water–nitrogen interaction had no

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significant effect on the pH in these two layers. The nitrogen application rate significantly influenced the pH in the 40–60 cm soil layer ($P < 0.05$). The soil electrical conductivity (EC) in the 0–20 cm layer ranged from 374.8 to 507.77 µS cm^{-1}, which first increased but then decreased with increasing nitrogen application rate. Both the amount of irrigation and the water–nitrogen interaction significantly influenced the EC ($P < 0.001$). Under the W1 condition, compared with the N1 treatment, the N2 treatment resulted in a significant increase of 24.9%. Compared with those in the N1 treatment, the N2 and N3 treatments in the W2 condition significantly increased by 25.9% and 36.1%, respectively.

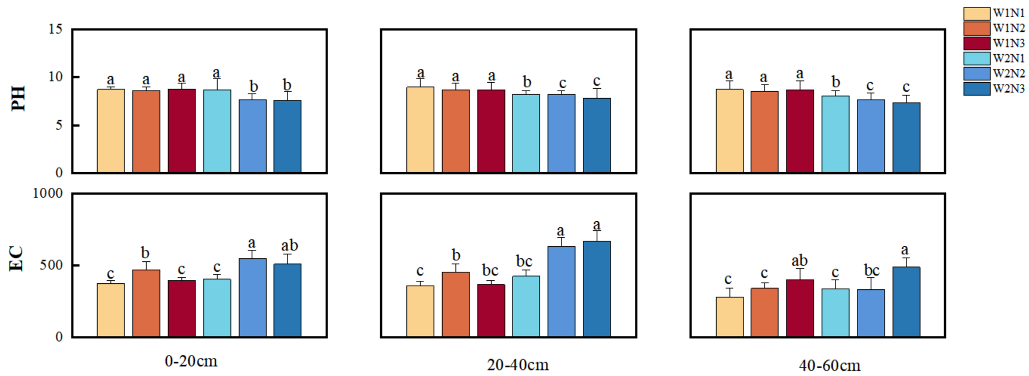

Figure 6. *Soil moisture content of different soil layers under different water–nitrogen coupling treatments. Different letters in each column indicate that the difference between different nitrogen treatments under the same irrigation treatment conditions was significant (P < 0.05). The error bars represent ± SDs*

Table 5. *Analysis of variance the effects of water–nitrogen coupling on soil pH and electrical conductivity in different soil layers*

ANOVA						
	pH			EC		
Soil depth（cm)	**0-20**	**20-40**	**40-60**	**0-20**	**20-40**	**40-60**
W	***	***	***	**	***	ns
N	**	ns	ns	***	**	***
W*N	**	ns	ns	**	ns	ns

ns denotes not significant; * denotes a significant correlation at the P < 0.05 level; ** denotes a significant correlation at the P < 0.01 level; and *** denotes a significant correlation at the P < 0.001 level

Effects of water–nitrogen coupling on the dynamic changes in the plant height and basal stem diameter of Sophora alopecuroides

As shown in *Figure 7*, the different irrigation rates and nitrogen application rates affected the plant height and stem diameter. Across all the treatments, both the plant height and stem diameter first increased but then gradually stabilized as the growth period progressed, following a consistent growth pattern. With increasing temperature from July to August, *S. alopecuroides* grew rapidly, and the plant height and stem diameter reached their maximum values in early September (harvest stage)., respectively, than those under W1.

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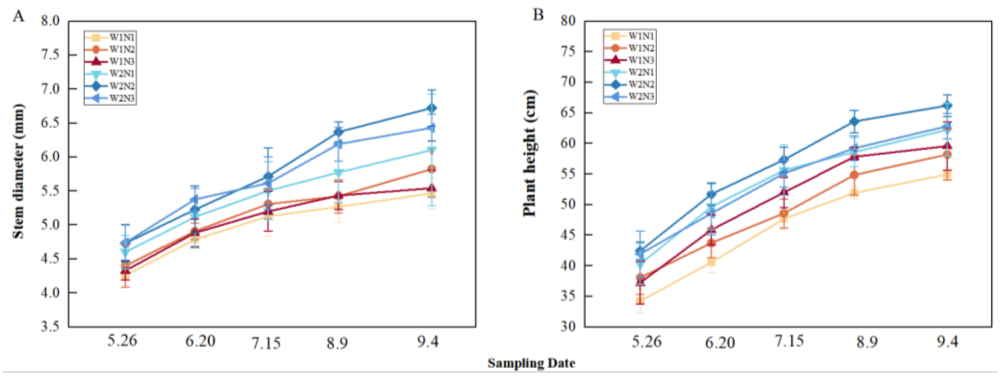

Figure 7. *Dynamic changes in the plant height and stem diameter of Sophora alopecuroides. Error bars represent ± SD*

Effects of water–nitrogen coupling on dry matter accumulation and distribution in Sophora alopecuroides

As indicated in *Table 6*, with the progression of the growth period, dry matter accumulation exhibited a single-peak curve, with all the treatments reaching a peak in mid-to-late August. Irrigation significantly affected ($P < 0.05$) the aboveground dry matter accumulation in S. alopecuroides across all the growth stages, whereas nitrogen application had a significant effect ($P < 0.05$) at the flowering and harvest stages, and the interaction between water and nitrogen had a significant effect ($P < 0.05$) only at the harvest stage. At the harvest stage (early September), the total dry matter accumulation increased with increasing nitrogen application rate. Under the N1 and N2 conditions, W2 significantly increased by 18.7% and 20.5%, respectively, compared with N1, whereas under the N3 condition, no significant differences were observed among all the treatments.

Table 6. *Distribution of dry matter in Sophora alopecuroides under the different water–nitrogen coupling treatments*

Treatment	Fertile period			
	Vegetative period	Anthesis	Fruiting period	Harvest period
W1N1	3.6 ± 0.40c	6.87 ± 0.42b	9.83 ± 0.44d	9.64 ± 0.53b
W1N2	4.15 ± 0.69c	8.27 ± 0.83a	10.80 ± 0.47c	10.35 ± 0.74b
W1N3	4.43 ± 0.36bc	8.09 ± 0.68a	11.33 ± 0.82bc	10.98 ± 0.69ab
W2N1	5.36 ± 0.52ab	8.64 ± 0.95a	11.88 ± 1.82bc	11.44 ± 1.51a
W2N2	6.05 ± 1.16a	9.08 ± 0.76a	13.17 ± 1.01a	12.47 ± 0.62a
W2N3	5.35 ± 1.10ab	9.74 ± 1.34a	12.96 ± 0.92a	12.55 ± 1.64a
ANOVA				
W	***	*	***	***
N	ns	*	ns	*
W×N	ns	ns	ns	*

The different letters in each column indicate that the difference between the different nitrogen treatments under the same irrigation treatment conditions was significant ($P < 0.05$). W-moisture; N-nitrogen fertilizer. ns denotes not significant; * denotes a significant correlation at the $P < 0.05$ level; ** denotes a significant correlation at the $P < 0.01$ level; and *** denotes a significant correlation at the $P < 0.001$ level. The error bars represent ± SDs

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As shown in *Figure 8*, under the different water–nitrogen coupling treatments, the distribution ratios of dry matter in the stems and leaves varied at the different stages. With the advancement of the growth period, the amount of dry matter accumulation in the stems and leaves first increased but then decreased. As the growth period progressed, the amount of dry matter accumulation in the stems gradually increased, and the proportions in the stems and leaves of the plants gradually became similar. At the vegetative stage, the amount of dry matter accumulation in the stems under the high-water W2 regime was significantly greater ($P < 0.05$) than that under the W1 regime, but with increasing nitrogen application rate, there was no significant difference among the treatments. At the fruiting stage, the amount of leaf dry matter accumulation under each treatment peaked.

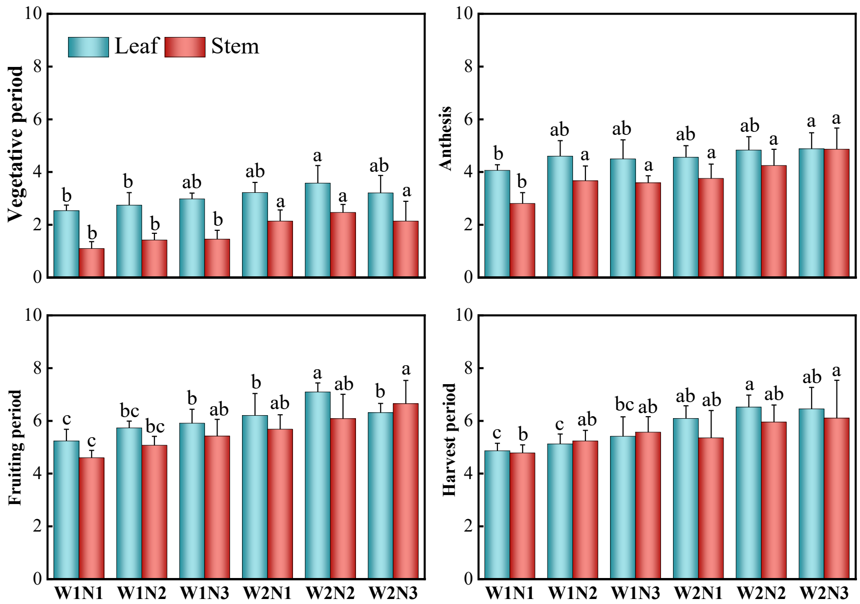

Figure 8. *Effects of the different water–nitrogen coupling treatments on the distribution of dry matter in Sophora alopecuroides beans. The different letters in each column indicate that the difference between the different nitrogen treatments under the same irrigation treatment conditions was significant (P < 0.05). The error bars represent ± SDs*

Effects of water–nitrogen coupling on the yield and water–fertilizer use efficiency of Sophora alopecuroides

As indicated in *Table 7*, the fresh grass yields ranged from 2370.4 to 4014.7 kg hm^{-2}. Irrigation and nitrogen application significantly affected the yield of *S. alopecuroides* ($P < 0.01$), whereas the interaction between water and nitrogen had no significant effect. Irrigation, nitrogen application, and their interaction significantly influenced the IWP ($P < 0.001$). Under reduced irrigation (W1), the irrigation water productivity (IWP) was greater than that under conventional irrigation (W2). Under W1 conditions, the IWP value under N3 increased significantly by 25.4% and 16.9% compared with that under N1 and N2, respectively. Under W2 conditions, compared with those under N1 and N3, the IWP under N2 increased significantly (by 14.01% and 2.7%, respectively). In the N1–N3 treatments, the IWP values under W1 increased significantly by 29.6%, 21.4%, and 46.7%, respectively, compared with those under W2. Compared with those in the W1N2, W1N3, and W2N3 treatments, nitrogen partial factor productivity (NPP) in the W2N2 treatment significantly

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increased by 61.8%, 33.15%, and 54.3%, respectively. Nitrogen application, irrigation, and their interaction significantly influenced the N content in *S. alopecuroides* ($P < 0.01$).

Table 7. *Effects of the different water–nitrogen coupling treatments on the yield and irrigation water use efficiency*

Treatment	Yield (kg hm^{-2})	Irrigation water productivity (kg m^{-3})	Nitrogen partial productivity (kg kg^{-1})
W1N1	3.6 ± 0.40c	6.87 ± 0.42b	9.83 ± 0.44d
W1N2	4.15 ± 0.69c	8.27 ± 0.83a	10.80 ± 0.47c
W1N3	4.43 ± 0.36bc	8.09 ± 0.68a	11.33 ± 0.82bc
W2N1	5.36 ± 0.52ab	8.64 ± 0.95a	11.88 ± 1.82bc
W2N2	6.05 ± 1.16a	9.08 ± 0.76a	13.17 ± 1.01a
W2N3	5.35 ± 1.10ab	9.74 ± 1.34a	12.96 ± 0.92a
ANOVA			
W	***	***	***
N	**	**	***
W×N	ns	*	***

The different letters in each column indicate that the difference between the different nitrogen treatments under the same irrigation treatment conditions was significant (P < 0.05). W-moisture; N-nitrogen fertilizer. ns denotes not significant; * denotes a significant correlation at the P < 0.05 level; ** denotes a significant correlation at the P < 0.01 level; and *** denotes a significant correlation at the P < 0.001 level. The error bars represent ± SDs

Effects of water–nitrogen coupling on the total alkaloid content in Sophora alopecuroides

As shown in *Figure 9*, the total alkaloid content in *Sophora alopecuroides* tended to vary among the treatments: W1N1 and W2N3 tended to initially increase and subsequently decrease, whereas W1N2, W1N3, W2N1, and W2N2 tended to increase gradually. At the harvest stage, the total alkaloid content increased with increasing nitrogen application rate. The total amount of alkaloid accumulation in the whole plant ranged from 7.99 to 16.15 mg·plant^{-1}, and it gradually increased with increasing nitrogen application rate under all the treatments. Compared with that in the N1 treatment group, the total alkaloid accumulation in the N2 and N3 treatment groups significantly increased by 102.1% and 65.5% ($P < 0.05$), respectively. Moreover, compared with that in the N1 treatment group, the total alkaloid accumulation in the N3 and N2 treatment groups significantly increased by 40.5% and 17.4% ($P < 0.05$), respectively. Under the N1–N3 treatment conditions, no significant difference in total alkaloid accumulation was observed between the W1 and W2 treatment groups.

Correlations among various indicators of Sophora alopecuroides

For deep-rooted plants such as *Sophora alopecuroides L.*, which exhibit distinct vertical stratification in terms of their root architecture, analyzing soil data aggregated across the entire profile is inadequate. This approach obscures the stratum-specific responses of the root system to edaphic factors and negates the ability to elucidate the mechanisms underlying root-environment interactions, which are inherently spatially heterogeneous. As shown in *Figure 10,* yield was significantly positively correlated with plant height, stem diameter, and dry matter accumulation ($P < 0.001$). There was no

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significant correlation between the alkaloid content and plant height, stem diameter, or dry matter accumulation, whereas alkaloid accumulation was significantly positively correlated with dry matter accumulation ($P < 0.01$).

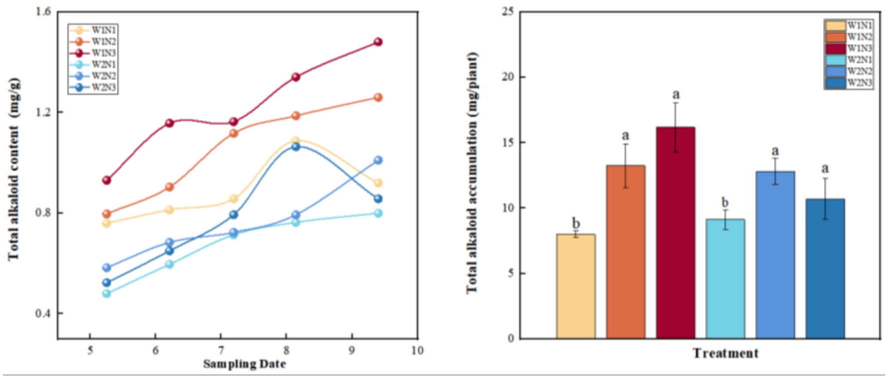

Figure 9. *Effects of the different water–nitrogen coupling treatments on the content and accumulation of alkaloids in Sophora alopecuroides beans. The different letters in each column indicate that the difference between the different nitrogen treatments under the same irrigation treatment conditions was significant (P < 0.05). The error bars represent ± SDs*

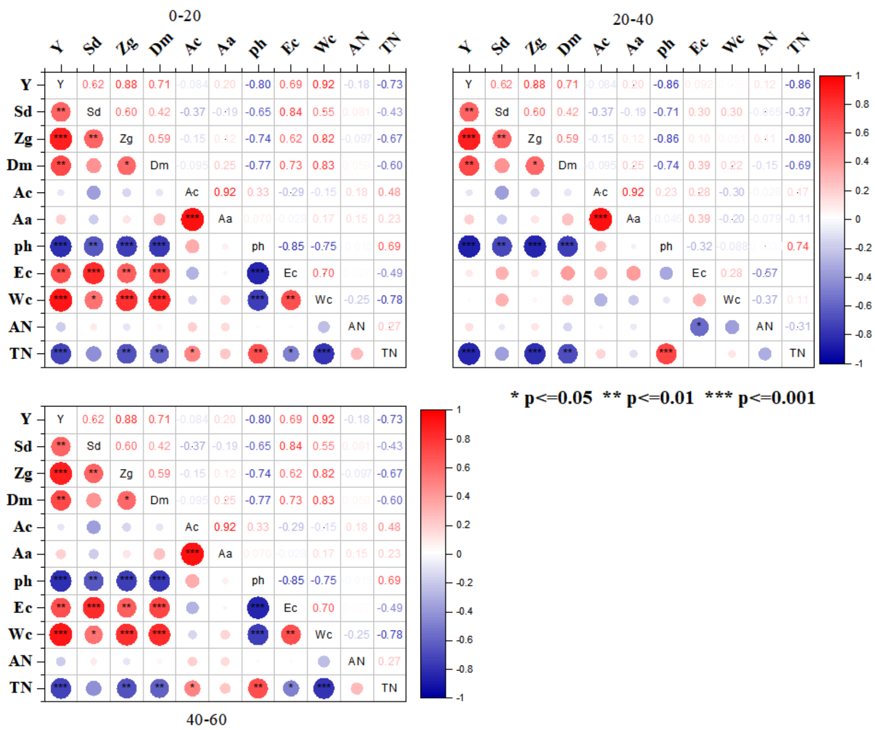

Figure 10. *Correlations between plant indicators. The different letters in each column indicate that the difference between the different nitrogen treatments under the same irrigation treatment conditions was significant (P < 0.05). W-moisture; N-nitrogen fertilizer. * denotes a significant correlation at the P < 0.05 level; ** denotes a significant correlation at the P < 0.01 level; and *** denotes a significant correlation at the P < 0.001 level. Y: Yield; Sd: Stem diameter; Zg: Plant height; Dm: Dry matter mass; Ac: Alkaloid content; Aa: Alkaloid accumulation; Ph: Acidity/alkalinity (pH); Ec: Electrical conductivity; Wc: Water content; AN: Nitrate- N; TN: Total nitrogen*

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In the 0–20 cm soil layer, the pH was extremely significantly negatively correlated with the yield, stem diameter, electrical conductivity (EC), and soil moisture content ($P < 0.001$). The soil moisture content was significantly positively correlated with yield, stem diameter, and dry matter accumulation ($P < 0.001$) and significantly positively correlated with plant height ($P < 0.01$). The total nitrogen content was significantly positively correlated with the alkaloid content ($P < 0.05$). In the 20–40 cm soil layer, the pH was significantly negatively correlated with yield, plant height, stem diameter, dry matter accumulation, EC, and soil moisture content ($P < 0.001$), whereas the EC was significantly positively correlated with dry matter accumulation ($P < 0.01$). In the 40–60 cm soil layer, the pH was significantly negatively correlated with yield, plant height, stem diameter, and dry matter accumulation ($P < 0.001$), whereas the EC and soil moisture content were not significantly correlated with yield, plant height, stem diameter, or dry matter accumulation.

Discussion

Effects of water–nitrogen coupling on the soil environment for Sophora alopecuroides growth

Soil serves as the material carrier for plant growth, and the optimization of its physical and chemical properties strongly affects the physiological metabolism of plants (Wang et al., 2024). This study revealed that the amount of irrigation and nitrogen application rate significantly increased the soil moisture content in the 0–40 cm soil layer. This increase occurred because high-irrigation conditions directly increase the soil moisture content. In addition, nitrogen application can increase the water-holding capacity of soil by affecting the level of soil microbial activity and soil structure. Moreover, the application of nitrogen fertilizer promotes the growth of *Sophora alopecuroides*, and more luxuriant vegetation reduces water evaporation, ultimately leading to an increase in the soil moisture content (Zhou et al., 2023; Zhang et al., 2024). The interaction between water and nitrogen significantly influenced only the soil moisture content in the 20–40 cm soil layer. This effect may have occurred because the active root zone of *Sophora alopecuroides* is located in this soil layer. Water–nitrogen coupling regulates root water absorption efficiency, nutrient migration, and the rhizosphere microenvironment, thus increasing the moisture content in this layer, which is sensitive to water–nitrogen interactions. In the 0–20 cm soil layer, the soil moisture content was strongly significantly positively correlated with plant height, stem diameter, and dry matter accumulation in *Sophora alopecuroides* ($P < 0.001$), indicating that surface soil moisture is a key factor driving the aboveground growth of this species.

Irrigation and nitrogen application reduced the pH of the 0–20 cm soil layer. Under the high-moisture treatment (W2), the pH values under the N2 and N3 treatments were significantly lower than those under the other treatments; this may have occurred because excessive irrigation and nitrogen application altered the ion balance in the soil. Nitrogen fertilizers produce acids through nitrification, whereas water regulates the intensity of acidification and the leaching of base ions by affecting microbial activity and leaching processes, thereby promoting the accumulation of acidic substances in the soil (Gu et al., 2015; Zhang et al., 2022), ultimately leading to a decrease in the soil pH. The soil EC was significantly affected by the interaction between water and nitrogen. In the 0–40 cm soil layer, the soil EC significantly increased under high-moisture conditions, primarily because water promoted ion dissolution and the enrichment effect caused by deep leaching.

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Moreover, the soil pH was extremely significantly negatively correlated with the aforementioned growth indices ($P < 0.001$), possibly because an alkaline soil environment affects the efficiency of root nutrient absorption, thereby limiting plant growth (Zhou, 2024). The correlation between the EC and dry matter accumulation varied in the different soil layers: the EC in the 0–20 cm soil layer was significantly positively correlated with dry matter accumulation ($P < 0.05$), whereas only a weak correlation was observed in the 20–40 cm soil layer; this may occur because when the salt content in surface soil increases moderately, it stimulates plants to activate osmotic adjustment mechanisms, thereby promoting dry matter accumulation to cope with stress (Ma, 2024); however, as the soil layer deepens, the buffering capacity of roots against salt increases, and the direct impact of salt stress decreases.

Effects of water–nitrogen coupling on the growth and yield of Sophora alopecuroides

The height and stem diameter of *Sophora alopecuroides* first increased but then stabilized as the growth period progressed, which is consistent with the growth patterns of most herbaceous plants (Yin, 2024). Under the high-water treatment (W2), the use of medium and high nitrogen levels (N2 and N3, respectively) promoted the growth of *Sophora alopecuroides* in terms of plant height and stem diameter. On the one hand, an adequate water supply ensures turgor pressure and elongation of plant cells, which is beneficial for the formation of the morphological structure of medicinal plants (Xing et al., 2022); on the other hand, nitrogen, a key element for plant growth, can provide more energy and substances for the growth of *Sophora alopecuroides*. Moreover, under reduced irrigation (W1), the medium- and high-nitrogen treatments (N2 and N3, respectively) significantly increased plant height and stem diameter, indicating that nitrogen can alleviate the inhibitory effect of water stress on growth (Chen et al., 2024). This study revealed that the dry matter weight under each treatment first increased but then decreased with increasing growth period, and the proportions of aboveground dry matter in the stems and leaves gradually became similar. These characteristics may be related to the aging and abscission of *Sophora alopecuroides* leaves due to the decrease in temperature during the late growth stage (Wen et al., 2019). Compared with those in the low-water treatment (W1), the plant height, stem diameter, and dry matter accumulation in the high-water treatment (W2) were significantly greater, indicating that water is among the main factors affecting the morphological development and dry matter accumulation of *Sophora alopecuroides*.

The fresh grass yield of biennial *Sophora alopecuroide*s varied significantly under the different water and nitrogen treatments. Under the W1 regime (limited water), the yield increased with increasing nitrogen application rate, whereas under the W2 regime (sufficient water), the yield first increased but then decreased. These findings indicate that under adequate water conditions, excessive nitrogen application may lead to the imbalanced growth of Sophora alopecuroides and thus reduce its yield.

Under W2 (sufficient water), both the nitrogen partial productivity (NPP) and the irrigation water productivity (IWP) reached their highest levels in the N2 treatment but slightly decreased in the N3 treatment. This observation is consistent with the response pattern of the yield under W2, which once again confirms that high nitrogen application may reduce the use efficiency of resources (water and nitrogen) when the amount of water is sufficient. The nitrogen application rate, amount of irrigation, and their interaction strongly significantly influenced the NPP ($P < 0.001$), which demonstrates that the NPP highly depends on the balance between the water and nitrogen supplies. In field

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production, high nitrogen application under conventional irrigation conditions should be avoided, as it not only fails to significantly increase yields but also significantly reduces the IWP and NPP, resulting in nitrogen fertilizer waste and potential environmental risks. There was an extremely significant positive correlation between yield and plant height, stem diameter, and dry matter accumulation ($P < 0.001$), indicating that the better the growth and development of Sophora alopecuroides and the better its various growth indicators, the higher the harvested yield (Wang, 2024).

Effects of water–nitrogen coupling on alkaloid accumulation in Sophora alopecuroides

Alkaloids are a large class of nitrogen-containing organic compounds; they are among the types of secondary metabolites formed by plants through interactions with the environment during long-term evolution and constitute important active components of *Sophora alopecuroides* (Pant et al., 2021). In this study, the total alkaloid content under the low-water treatment (W1) was greater overall than that under the high-water treatment (W2) throughout the growth period of *Sophora alopecuroides*. These findings are consistent with the conclusions of previous studies showing that moderate drought stress is beneficial for the synthesis and accumulation of plant alkaloids (Dirk and Maik, 2013; Dursun and Ahmet, 2021; Liu et al., 2017). Drought stress may cause the activation of secondary metabolic pathways in *Sophora alopecuroides*, prompting the plant to synthesize more alkaloids to cope with adverse conditions. The alkaloid content represents only a relative amount and cannot indicate the alkaloid yield level. The use of total alkaloid accumulation to represent the absolute alkaloid yield per plant is more intuitive and accurate. Nitrogen application significantly affected the total alkaloid accumulation in *Sophora alopecuroides*, and the alkaloid accumulation under each treatment increased significantly with increasing nitrogen application rate; this occurs because nitrogen is an important component of alkaloids, and a sufficient nitrogen supply provides the material basis for the synthesis of alkaloids. Moreover, nitrogen application may promote the synthesis and transformation of alkaloid precursor substances by regulating the carbon–nitrogen metabolic balance in plants (Zhang et al., 2019).

Conclusion

During the growth period, the 40% reduction in irrigation (W1, 1080 m^3 hm^{-2}) coupled with high nitrogen application (N3, 210 kg hm^{-2}) effectively increased the total alkaloid content in Sophora alopecuroides throughout the growth period while increasing irrigation water productivity (2.86 kg m^{-3}) and significantly promoting alkaloid accumulation per plant (16.15 mg of $plants^{-1}$), laying a foundation for improving quality. In conclusion, the optimal water–nitrogen coupling mode throughout the growth period is the application of reduced irrigation (W1, 1080 m^3 hm^{-2}) combined with a high nitrogen application rate (N3, 210 kg hm^{-2}), which can effectively provide technical support and a theoretical basis for the artificial high-quality cultivation of S. alopecuroides in arid regions.

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