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Assessing the relative importance of species choice, irrigation, and nitrogen management for sustainable tropical forage legume systems

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Abstract

Water scarcity and declining soil fertility are placing increasing pressure on forage-based livestock systems in the tropics. Although irrigation and nitrogen inputs are widely used to improve forage accumulation, their relative importance compared with species choice remains unclear for tropical forage legumes. This field study examined the effects of irrigation level (60% (I60), 80% (I80), and 100% (I100) of crop water requirement), fertilizer management (organic manure 30 t ha⁻¹, 100 kg ha⁻¹ urea, and 300 kg ha⁻¹ urea), and three forage legume species: *Desmodium uncinatum* (Jacq.) DC. (ILRI 6765), *Stylosanthes scabra* Vogel (ILRI 140), and *Stylosanthes hamata* (L.) Taub. (ILRI 75) on forage accumulation, water use efficiency (WUE), and nutritive value. The experiment followed a split-split plot design with three replications. Data was collected over two years (2021–2022). No significant two or three-way interactions on forage accumulation and WUE were found, indicating that the effects of irrigation, fertilizer, and species acted largely independently. Increasing irrigation raised forage accumulation from 2.8 t ha⁻¹ under I60 to 4.1 t ha⁻¹ under full irrigation (I100), but no significant effect on WUE or nutritive value. Fertilizer application did not significantly influence forage accumulation, WUE, or nutritive value, suggesting limited response of legumes to added nitrogen. In contrast, significant differences were observed among species. *D. uncinatum* ILRI 6765 consistently showed the highest WUE (9.5 kg ha⁻¹ mm⁻¹) and nutrient-based water productivity, while *S. hamata* ILRI 75 showed lower efficiency despite relatively high crude protein content. Irrigation at 80% of crop water requirement provided the best balance between forage accumulation, water use efficiency, and nutritive value. These results show that, under sub-humid tropical conditions, species choice plays a more important role than irrigation or nitrogen input in determining forage legume performance. Focusing on well-adapted, efficient legume species may contribute to improving forage sustainability under increasing water constraints.

Keywords Deficit irrigation, Forage accumulation, Forage legumes, Nutritive value, Sustainable intensification, Water use efficiency



1 Introduction

Sustainable livestock production systems are critical to ensuring food security while safeguarding environmental integrity. High quality forage underpins the productivity and ecological resilience of mixed farming systems worldwide, acting as the foundation that links agricultural output with natural resource conservation [1]. As global challenges such as population growth, land degradation, and shifting climate patterns intensify, the demand for resilient and sustainable forage production systems has become increasingly critical.

Across Africa, livestock and mixed farming systems are central to rural livelihoods, providing food, income, and ecosystem services for millions of households. However, the continent faces intensifying pressures from recurrent droughts, erratic rainfall, and increasing competition for limited water resources [2, 3]. These stresses have direct consequences for forage systems, with water scarcity and declining soil health threatening the accumulation, nutritive value, and reliability of forage supply, particularly in semi-arid and sub-humid regions.

In East Africa, livestock dependent communities are particularly vulnerable to climate induced forage insecurity. Livestock sector growth is constrained by diminishing natural resources and the need to produce more with fewer inputs, yet evidence based solutions for optimizing forage systems in these contexts remain limited [4]. In Ethiopia, livestock is the backbone of the national economy and food system, supporting the majority of rural livelihoods. However, forage shortages, land competition, and increasing water scarcity exacerbated by climate change pose substantial threats to the sustainability and productivity of mixed farming systems [5, 6]. Ensuring resilient, high yielding and resource efficient systems is therefore a national priority for agricultural development.

A central challenge in these water-limited environments is improving forage accumulation and water use efficiency (WUE), defined as the amount of biomass produced per unit of water used [7, 8]. Although substantial research has addressed the optimization of irrigation and fertilizer use in forage grasses [5, 9, 10], their effects in forage legume systems remain underexplored, particularly under field conditions. Forage legumes offer distinct advantages for addressing these sustainability challenges [11]. Their symbiotic relationship with nitrogen fixing bacteria contributes to improved soil fertility, reducing dependence on synthetic fertilizers and supporting ecological sustainability [12]. The deep-rooted architecture of forage legumes supports drought tolerance, enabling better access to soil moisture and potentially improving WUE. In mixed pastures, legumes also enhance forage nutritive value and stabilize forage accumulation [13].

A critical knowledge gap remains regarding the optimal management of irrigation and fertilizer for forage legumes under Ethiopian conditions. Most existing studies focus on grass species, and the unique physiological traits of legumes particularly nitrogen fixation and growth phenology limit the direct transferability of these findings. Moreover, empirical data are lacking on how different irrigation regimes and fertilizer rates interact to influence forage accumulation, WUE, and forage nutritive value across diverse forage legume species under field conditions in Ethiopia. In addition, practical recommendations for irrigation and fertilizer management in Ethiopian legume systems are constrained by limited field-based evidence.

This study examines how varying irrigation regimes and fertilizer rates affect forage accumulation, water use efficiency (WUE), and forage nutritive value in different forage

legume species under Ethiopian field conditions. We hypothesize that: (i) moderate deficit irrigation can improve WUE without substantially compromising forage accumulation; (ii) balanced fertilizer application enhances both forage accumulation and forage nutritive value; and (iii) interactions between irrigation and fertilization will reveal species-specific responses that inform targeted management strategies.

The main objective of this study is to generate robust field based evidence to guide the sustainable management of legume based forage systems in water-limited environments. Specifically, the study aims to: (i) quantify the effects of varying irrigation regimes on forage accumulation, water use efficiency, and forage nutritive value in multiple forage legume species; (ii) assess the impact of different fertilizer application rates on forage accumulation, WUE, and forage nutritive value; and (iii) examine the interaction between irrigation and fertilizer inputs in regulating these outcomes, thereby identifying species-specific management recommendations suitable for the Ethiopian context.

By providing high quality, locally relevant data and analysis, this research contributes to the development of improved water and nutrient management practices that enhance forage productivity and resource use efficiency. The findings offer insights for both policy and practice, supporting climate smart livestock production in Ethiopia and similar agro-ecological settings.

2 Materials and methods

2.1 Study area description

The field experiment was conducted in Robit Bata Kebele, located in the Bahir Dar Zuriya District of the Amhara Region, Ethiopia (Fig. 1). The site is part of the Lake Tana basin and is situated about 20 km north of Bahir Dar at an elevation of roughly 1,851 m. The area is characterized by a sub-humid climate, receiving an average annual rainfall of about 1,444 mm based on two decades of records. Rainfall follows a unimodal pattern,

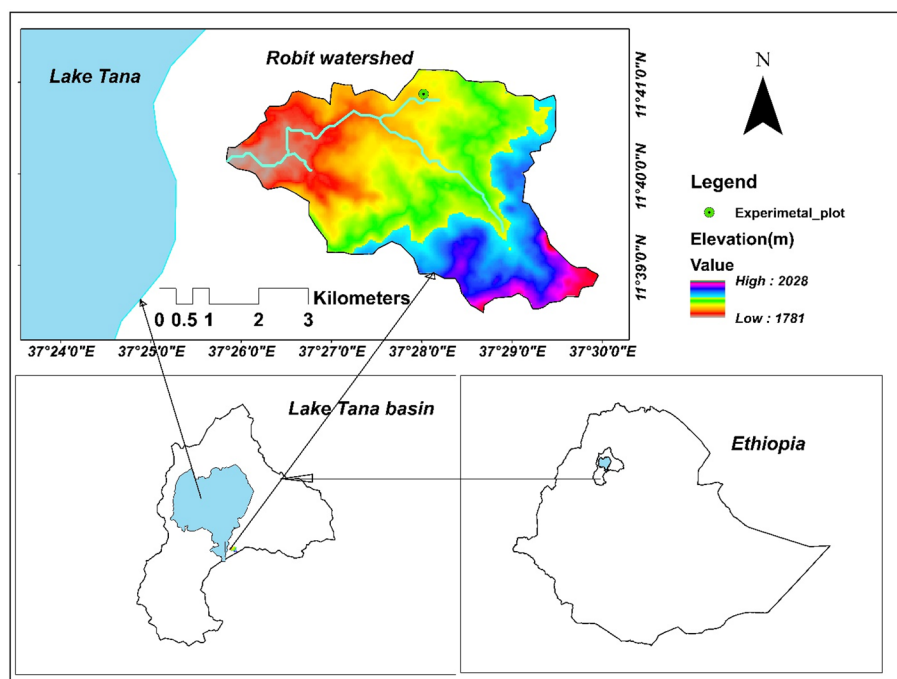


Fig. 1 Location map of the study site in the Robit Watershed, Lake Tana Basin, Ethiopia

with the main rainy season occurring from June to September. Mean monthly temperatures vary from 8.1 °C to 30.3 °C, with long-term averages of 12.5 °C for minimum and 27.5 °C for maximum temperatures. The area typically experiences around 8 h of sunshine per day, 58% relative humidity, and an average wind speed close to 0.82 m s⁻¹.

Farming in the community is predominantly rain-fed and based on mixed crop-livestock systems, complemented by small-scale irrigation used mainly during the dry season for vegetable production [14]. Major rain-fed crops include maize (*Zea mays* L.), finger millet (*Eleusine coracana* (L.) Gaertn.), tef (*Eragrostis tef* (Zucc.) Trotter), and barley (*Hordeum vulgare* L.), while tomato, onion, potato, cabbage, pepper, and garlic are commonly grown under irrigation. Livestock feeding is largely dependent on natural pastures and crop residues, which generally provide low-nutritive value forage.

2.2 Experimental design and treatments

The experiment was arranged in a split-split plot design within a randomized complete block design, with three replications (blocks). Irrigation regimes were assigned to main plots, fertilizer to subplots, and forage species to sub-sub plots. The sub-sub plot size was 9 m², with 1 m buffer spaces maintained between adjacent plots. In total, 81 experimental plots were established.

2.2.1 Forage species

Three forage legumes were evaluated: *Desmodium uncinatum* (Jacq.) DC. (accession ILRI 6765), *Stylosanthes scabra* Vogel (accession ILRI 140), and *Stylosanthes hamata* (L.) Taub. (accession ILRI 75). Seeds of the three forage legumes genotypes were obtained from the International Livestock Research Institute (ILRI) forage genebank in Addis Ababa, Ethiopia. Planting was carried out in July 2020 at the onset of the rainy season, ensuring uniform establishment across all plots. The irrigation treatments started after the first harvest at the end of the rainy season. The experiment was repeated over two year i.e. during irrigation seasons of 2020/2021 and 2021/2022.

2.2.2 Soil water content and irrigation management

Three irrigation levels were applied, corresponding to 60% (I60), 80% (I80), and 100% (I100) of the total available soil water content. The amount of irrigation water required for each plot under full irrigation (I100) was calculated using Eq. 1 [15, 16]. For I60 and I80, 60% and 80% of the full irrigation volume calculated for I100 were applied, respectively.

$$I_n = \frac{(F_c - \theta_i) \times D \times A}{100} \quad (1)$$

where I_n is the volume of irrigation water (m³), F_c is the soil field capacity, θ_i is the measured soil moisture before irrigation, D is the effective rooting depth (m), A is the surface area of the sub-sub plot (9 m²).

Irrigation water was applied individually to each sub-sub plot using 15-L graduated watering cans. The water was carefully measured according to the calculated requirement, and trained personnel ensured as uniform application as possible along the rows within each plot. To prevent runoff, soil bunds were constructed around each plot. The

total amount of irrigation water applied to each treatment over the entire growing season was recorded and is provided in Table 1.

Soil moisture was monitored to a depth of 1 m at six depths (10, 20, 30, 40, 60, 100 cm) using a PR2/6 soil moisture profiler (Delta-T Devices, UK). One access tube was installed in the center of each sub-subplot. Measurements were taken weekly as well as before planting and at each harvest. The PR2/6 soil moisture readings were calibrated using soil samples collected from ten representative plots covering a range of moisture conditions. Separate calibration equations were developed for 10, 20, and 30 cm depths, and a single equation was used for 40–100 cm. This ensured accurate estimation of soil volumetric water content.

2.2.3 Fertilizer management

Three fertilizer levels were applied: 30 t ha⁻¹ organic manure, 100 kg ha⁻¹ urea, and 300 kg ha⁻¹ urea. The manure rate represents typical farmer practice in the study area, while the 100 kg ha⁻¹ urea level corresponds to national fertilizer recommendations [17]. The higher rate of 300 kg ha⁻¹ urea was included to examine the response of forage accumulation and water productivity and nutritive value under intensified nitrogen supply. The fertilizer treatments represented a range of application rates commonly used under local management practices.

2.3 Crop management and harvesting

Plots were manually weeded to prevent competition with forage legumes, and no chemical pest or disease control measures were required during the experiment. The forage legumes were harvested once each year for this experiment at approximately 50% flowering. Harvesting was carried out in both experimental years (2020/2021 and 2021/2022). At each harvest, the fresh forage accumulated from the entire plot was weighed. A representative subsample of approximately 500 g was collected from each plot, oven-dried at 65 °C to constant weight, and used to determine dry matter (DM) content. Total forage accumulation per hectare was then estimated by multiplying fresh forage accumulation by DM fraction.

2.4 Forage nutritive value analysis

Representative dried forage subsamples from each treatment plot were analyzed for nutritive value parameters. Crude protein (CP) concentration, neutral detergent fiber

Table 1 Table 1. Total irrigation water applied (mm) plus effective rainfall across treatments during the 2020/2021 and 2021/2022 irrigation seasons

Forage species	Fertilizer	Irrigation level					
		I60	I80	I100	I60	I80	I100
		2020/2021			2021/2022		
<i>D. uncinatum</i> ILRI 6765	Manure	284.65	340.65	399.99	332.57	407.57	477.57
	Urea 100	298.99	363.65	428.32	372.57	457.57	534.24
	Urea 300	313.65	380.99	448.32	367.57	444.24	524.24
<i>S. hamata</i> ILRI-75	Manure	278.32	334.99	389.99	365.91	444.24	527.57
	Urea 100	300.32	364.32	426.65	367.57	449.24	532.57
	Urea 300	267.32	318.65	369.99	377.57	464.24	545.91
<i>S. scabra</i> ILRI-140	Manure	293.99	358.65	419.99	334.24	405.91	469.24
	Urea 100	305.65	368.65	434.99	359.24	434.24	520.91
	Urea 300	308.65	377.65	444.99	355.91	432.57	510.91

(NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), ash, In vitro digestibility of organic matter (IVDOM) and metabolizable energy (ME) content was analyzed in the animal nutrition laboratory of the International Livestock Research Institute (ILRI), Addis Ababa. Near-infrared reflectance spectroscopy (NIRS) was used with calibration equations developed for tropical forages. The calibration equations showed high predictive performance, with coefficients of determination (R^2) ranging from 0.82 to 0.99 and standard errors of cross-validation (SECV) ranging from 0.13 to 3.44 across parameters. Specifically, R^2 and SECV values were 0.98 and 3.44 for NDF, 0.99 and 1.62 for ADF, 0.94 and 0.92 for ADL, 0.91 and 0.82 for ash, 0.99 and 0.95 for IVDOM, and 0.99 and 0.13 for ME, based on the ILRI NIRS calibration database.

2.5 Water use efficiency and water productivity indices

In this study, water use efficiency (WUE) was expressed as the amount of above-ground dry forage accumulation produced per unit of crop evapotranspiration (ET) as shown in Eq. 2.

$$WUE(kgha^{-1}mm^{-1}) = Forage\ accumulation(Kgha^{-1})/ET(mm) \quad (2)$$

Evapotranspiration (ET) was estimated using the standard water balance method [5].

To compare treatment effects on forage nutritive value, a range of nutritive attributes was measured. Relative Feed Value (RFV) was calculated from Dry Matter Intake (DMI) and Digestible Dry Matter (DDM) following Rohweder et al., [18]:

$$RFV\ (relative\ feed\ value) = (DMI * \% DDM) / 1.29. \quad (3)$$

Where

$$DMI(dry\ mater\ intake) = 120/(\% NDF) \quad (4)$$

and

$$DDM\ (digestible\ dry\ mater) = 88.9 - (0.779 * \% ADF) \quad (5)$$

To account for both forage accumulation and nutritive value in estimating nutritional output, crude protein (CP) yield, RFV yield, and metabolizable energy (ME) yield were calculated as:

$$CP\ yield\ (kg\ CP\ ha^{-1}) = DM\ (kg\ ha^{-1}) \quad (6)$$

$$RFVyield\ (kg\ ha^{-1}) = DM\ (kg\ ha^{-1}) \times RFV\ (g\ g^{-1}) \quad (7)$$

Irrigation water productivity was also assessed not only for forage accumulation but also for nutrient and energy production. Water productivity in terms of crude protein (WP.CP) and metabolizable energy (WP.ME) was calculated as follows following Hussein et al., [6]:

$$WP.CP(gm^{-3}) = \frac{CP\ yield\ (g\ ha^{-1})}{Total\ water\ recieved\ by\ the\ crop\ (m^3ha^{-1})} \quad (8)$$

Irrigation water use efficiency (IWUE) in terms of metabolizable energy (ME)

$$WP.ME (MJm^{-3}) = \frac{ME \text{ yield } (MJ \text{ ha}^{-1})}{\text{Total water recieved by the crop } (m^3ha^{-1})} \quad (9)$$

2.6 Statistical analysis

Before running the statistical analyses, all response variables were checked for normal distribution using Q-Q plots and the Shapiro-Wilk test. A three-way and then two-way factorial ANOVA was then used to examine the effects of irrigation level, fertilizer treatment, and forage species (fixed effects), with replication/block treated as a random effect, on forage accumulation, water use efficiency (WUE), and nutritive value parameters. All statistical procedures, including ANOVA (Type II sums of squares) and post-hoc comparisons, were conducted in Python, primarily using the statsmodels library. A significance threshold of $p < 0.05$ was applied throughout, and pairwise differences among means were examined using Tukey's HSD.

3 Results

The significance levels for all treatment interactions and main effects are summarized in Table 2. When an interaction term was significant, the corresponding interaction means are reported; otherwise, only the relevant main effects are presented.

Where I is irrigation, F is fertilizer, FS is forage species, FA is forage accumulation, WUE is water use efficiency, CP is crude protein, NDF is neutral detergent fiber, ADF is acid detergent fiber, IVDOM is in vitro organic matter digestibility, ME is metabolizable energy, CPyield is crude protein yield, RFVyield is relative feed value yield, MEyield is metabolizable energy yield, WP.CP is water productivity in terms of crude protein and WP.ME is water productivity in terms of metabolizable energy and *** indicates $p < 0.001$.

3.1 Effect of irrigation, fertilizer, forage species and their interaction on forage accumulation

There was no three way as well as two way significant interaction effect of irrigation, fertilizer, and forage species on forage accumulation Table 2. This indicates that the effects of irrigation, fertilizer, and forage species on forage accumulation were largely independent.

Irrigation had a significant effect on forage accumulation ($p = 0.02$). Forage accumulation increased with increasing irrigation levels, with the lowest forage accumulation observed under the deficit irrigation of 60% available soil water (I60, 2.8 t ha⁻¹) while the highest forage accumulation was observed under full irrigation (I100, 4.1 t ha⁻¹) Fig. 2a.

Fertilizer application however had no significant effect on forage accumulation ($p > 0.05$). Application of urea at 100 kg ha⁻¹ produced the highest forage accumulation (3.83 t ha⁻¹), followed closely by organic manure at 30 t ha⁻¹ (3.53 t ha⁻¹). The highest urea rate of 300 kg ha⁻¹ resulted in a lower forage accumulation (3.21 t ha⁻¹) Fig. 2b.

Forage species had a significant effect on forage accumulation ($p = 0.02$). *D. uncinatum* ILRI 6765 produced the highest mean forage accumulation (3.92 t ha⁻¹), closely followed by *S. scabra* ILRI 140 (3.9 t ha⁻¹) Fig. 2c.

Table 2 Significance levels of irrigation, fertilizer, forage species and their interaction on the response variables

I	FA	WUE	CP	NDF	ADF	ADL	Ash	IVDOM	ME	CPyield	RFVyield	MEyield	WP:CP	WP:ME
	0.02	0.65	0.46	0.24	0.20	0.58	0.97	0.57	0.78	0.05	0.10	0.02	0.28	0.56
F	0.43	0.40	0.16	0.95	0.52	0.83	0.70	0.54	0.63	0.12	0.23	0.23	0.22	0.18
FS	0.02	0.012	***	***	***	***	***	***	***	0.01	0.00	0.04	0.01	0.03
IxF	0.89	0.98	0.04	0.43	0.48	0.31	0.77	0.77	0.81	0.69	0.74	0.86	0.78	0.88
I×FS	0.91	0.61	0.70	0.04	0.07	0.66	0.30	0.50	0.60	0.85	0.94	0.84	0.91	0.88
F×FS	0.76	0.51	0.16	0.08	0.09	0.26	0.17	0.54	0.67	0.86	0.89	0.74	0.83	0.71
I×F×FS	0.94	0.92	0.51	0.10	0.14	0.40	0.59	0.79	0.77	0.98	0.98	0.95	0.97	0.94

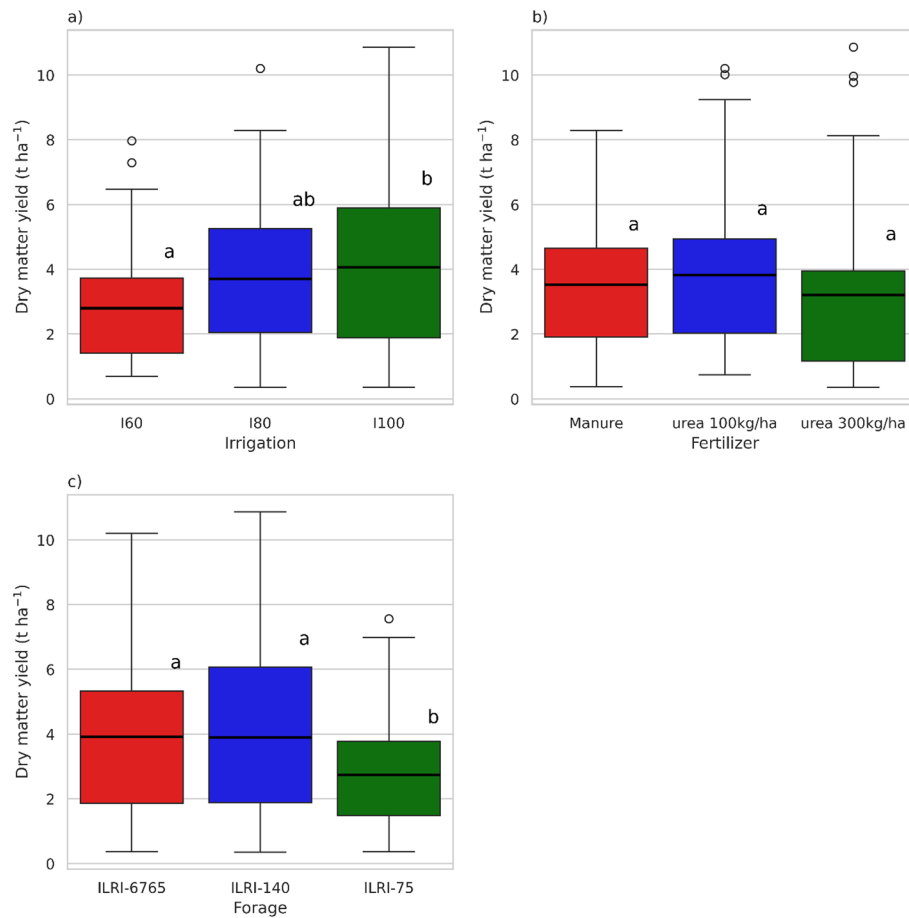


Fig. 2 Effect of irrigation (**a**), fertilizer (**b**) and forage species (**c**) on forage accumulation of three forage legume species (*D. uncinatum* ILRI 6765, *S. scabra* ILRI 140, and *S. hamata* ILRI 75) under three irrigation levels (I60, I80, I100) and fertilizer treatments in Robit watershed, Lake Tana basin, Ethiopia. Bars represent the mean $\pm$ standard error

3.2 Effect of irrigation, fertilizer, forage species and their interaction of WUE

No significant three way as well as two way interaction effects were observed among irrigation, fertilizer, and forage species, indicating that the factors influenced WUE independently within the range of conditions tested.

Water use efficiency was not significantly affected by the irrigation or fertilizer treatments (Table 2). Mean WUE values under the three irrigation levels were relatively similar, with I80 showing a slightly higher efficiency than both I60 and I100 Fig. 3a. The small numerical advantage at I80 likely reflects a more balanced soil moisture condition, where water was sufficient to support growth but not excessive enough to reduce efficiency through unnecessary water use. Fertilizer treatments followed the same pattern, with manure (8.91 kg ha⁻¹ mm⁻¹) and 100 kg ha⁻¹ urea (9.07 kg ha⁻¹ mm⁻¹) resulting in similar WUE values, while the highest urea rate (300 kg ha⁻¹) showed a slight reduction Fig. 3b. This decrease indicates that the higher nutrient supply does not proportionally translate into additional forage accumulation, thereby reducing efficiency.

In contrast, forage species had a significant ($p=0.012$) effect on WUE. *D. uncinatum* ILRI 6765 and *S. scabra* ILRI 140 showed markedly higher efficiencies compared to *S. hamata* ILRI 75 Fig. 3c. These differences may be attributed to inherent variations among species in their capacity to convert available water into forage accumulation. The

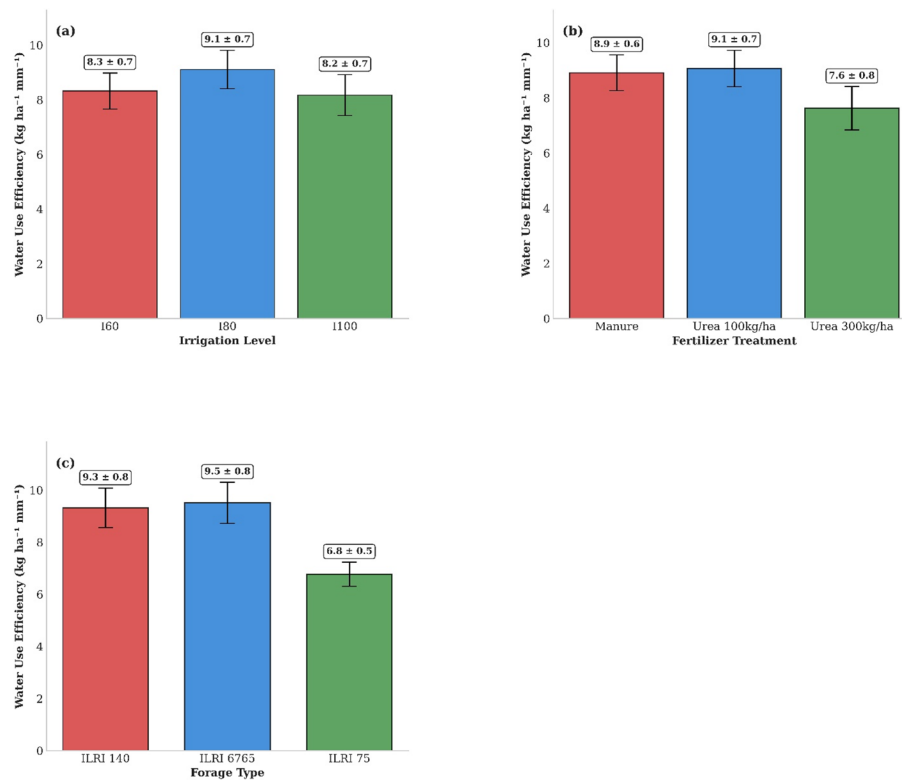


Fig. 3 Effect of irrigation (a), fertilizer (b), and forage species (c) on water use efficiency of three forage legume species (*D. uncinatum* ILRI 6765, *S. scabra* ILRI 140, and *S. hamata* ILRI 75) under three irrigation levels (160, 180, 1100) and fertilizer treatments in Robit watershed, Lake Tana basin, Ethiopia. Bars represent the mean $\pm$ standard error

higher-performing lines appeared to maintain better physiological responsiveness under the given soil moisture conditions, whereas *S. hamata* ILRI 75 likely produced less forage accumulation relative to water used, resulting in lower WUE.

3.3 Forage nutritive value parameters

There were no significant three-way or two-way interactions between forage, fertilizer, and irrigation for ash, ADF, ADL, IVDOM, and ME while there was a two way significant ($p = 0.04$) interaction effect of irrigation and fertilizer on CP as well as irrigation and forage species on NDF.

Although irrigation did not significantly affect any of the measured nutritional parameters, subtle differences were observed. The highest crude protein (CP) content was observed under I80, while the lowest was under I100 (Table 3). Similarly, IVDOM and ME showed minor variation across irrigation levels, with slightly higher IVDOM at I80 compared to I100, and ME following a similar pattern (Table 3). These patterns suggest that moderate irrigation may slightly enhance forage digestibility and energy content, likely due to improved plant physiological balance without excessive vegetative dilution.

Fertilizer type did not significantly influence any nutritional traits. Nonetheless, urea applied at 100 kg ha⁻¹ produced the highest CP and IVDOM, while urea at 300 kg ha⁻¹ resulted in slightly lower CP and ME (Table 3). Manure applications resulted in intermediate values for most parameters. These results indicate that within the applied ranges, forage nutritive value is relatively stable across nitrogen sources, although moderate mineral N may slightly enhance protein content and digestibility.

Table 3 Summary of nutritive value parameters for the three main treatments

Factor	Treatment	CP (%)	NDF (%)	ADF (%)	ADL (%)	Ash (%)	IVDOM (%)	ME (MJ/kg)
Forage	<i>S. scabra</i> ILRI 140	12.96 ± 2.14	63.1 ± 4.5	38.2 ± 3.2	5.8 ± 0.9	8.04 ± 1.78	52.5 ± 3.8	7.39 ± 0.57
	<i>D. uncinatum</i> ILRI 6765	14.51 ± 2.7	61.8 ± 3.9	36.5 ± 3.0	5.2 ± 0.8	10.58 ± 2.73	54.7 ± 2.7	7.51 ± 0.46
	<i>S. hamata</i> ILRI 75	14.83 ± 2.1	60.4 ± 4.1	35.8 ± 3.4	5.1 ± 0.7	8.03 ± 1.78	56.1 ± 3.4	7.99 ± 0.53
Fertilizer	Manure	13.68 ± 2.1	62.0 ± 4.2	37.0 ± 3.1	5.5 ± 0.8	8.75 ± 2.32	54.1 ± 4.0	7.61 ± 0.58
	Urea 100 kg/ha	14.51 ± 2.2	61.5 ± 3.8	36.6 ± 3.2	5.3 ± 0.7	8.77 ± 2.22	54.9 ± 3.4	7.70 ± 0.54
	Urea 300 kg/ha	14.11 ± 2.3	61.2 ± 4.1	36.2 ± 3.5	5.4 ± 0.8	9.14 ± 2.79	54.3 ± 3.5	7.58 ± 0.61
Irrigation	I60	14.17 ± 2.1	61.9 ± 3.9	36.7 ± 3.0	5.4 ± 0.7	8.85 ± 1.81	54.6 ± 3.4	7.65 ± 0.54
	I80	14.32 ± 2.3	61.6 ± 4.2	36.5 ± 3.1	5.3 ± 0.8	8.85 ± 3.07	54.8 ± 3.2	7.66 ± 0.56
	I100	13.81 ± 2.3	61.8 ± 4.0	36.8 ± 3.3	5.4 ± 0.8	8.96 ± 2.33	53.9 ± 4.3	7.58 ± 0.63

Forage species had a strong and highly significant influence on all nutritive value parameters ($P < 0.001$). Among the legumes, *S. hamata* ILRI 75 exhibited the highest CP, IVDOM, and ME, whereas *S. scabra* ILRI 140 had the lowest CP, IVDOM and ME (Table 3).

Overall, while irrigation and fertilizer had no statistically significant effect on forage nutritive value, the highest CP and IVDOM values were generally observed under I80 and with urea 100 kg ha⁻¹, suggesting that moderate water and nitrogen supply may slightly improve nutritional traits. However, the choice of forage species is the most important determinant of forage nutritive value, as evidenced by the consistent and significant differences.

3.4 Nutrient and energy yield

Irrigation had a significant effect on CP yield and ME yield but did not affect RFV yield (Table 2). The highest CP yield was recorded under I80, followed closely by I100, whereas I60 resulted in the lowest CP yield. The highest ME yield was observed for I100 while I60 had the lowest ME yield. The superior performance at I80 and I100 suggests that adequate but not excessive water availability supports both forage accumulation and nutrient synthesis.

Fertilizer did not significantly influence CP yield, RFV yield, or ME yield. Slightly higher CP and ME yield were observed for 100 kg ha⁻¹ urea compared with manure and 300 kg ha⁻¹ urea, but differences were small. These results reflect the limited fertilizer responsiveness of the forage legumes under conditions where soil nitrogen was not strongly limiting.

Forage species significantly influenced all nutrient-yield variables (Table 2). *D. uncinatum* ILRI 6765 produced the highest CP yield and RFV yield, while *S. hamata* ILRI 75 produced the lowest CP yield and RFV yield. ME yield followed the same pattern, with *D. uncinatum* ILRI 6765 reaching 28,221.51 MJ ha⁻¹ and *S. hamata* ILRI 75 producing the lowest mean value. These differences reflect the combined effect of forage accumulation and nutrient composition.

3.5 Water use efficiency in terms of nutrient production

Irrigation did not significantly affect water productivity in terms of crude protein (WP, CP) and metabolizable energy (WP,ME), although slightly higher mean values were

recorded at I80 compared with I100 and I60 Fig. 4a. This tendency suggests that moderate water supply may balance growth and physiological efficiency.

Fertilizer type also had no significant influence on nutrient-based water productivity. Both manure and 100 kg ha⁻¹ urea produced comparable values and tended to perform slightly better than 300 kg ha⁻¹ urea (Figs. 4b and 5b).

Water productivity expressed in terms of crude protein production (WP.CP) and metabolizable energy production (WP.ME) differed among forage species. *D. uncinatum* ILRI 6765 had the highest WP.CP, followed by *S. scabra* ILRI 140, whereas *S. hamata* ILRI 75 produced the lowest value. A similar trend was observed for WP.ME, where *D. uncinatum* ILRI 6765 again achieved the highest whereas *S. hamata* ILRI 75 had the lowest value Fig. 5c.

4 Discussions

4.1 Effect of irrigation, fertilizer, forage species and their interaction on forage accumulation

The marked increase in forage accumulation with increasing irrigation in this study aligns with fundamental plant-water relationships, and supports the prevailing understanding that water availability is a primary driver of forage accumulation in semi-humid

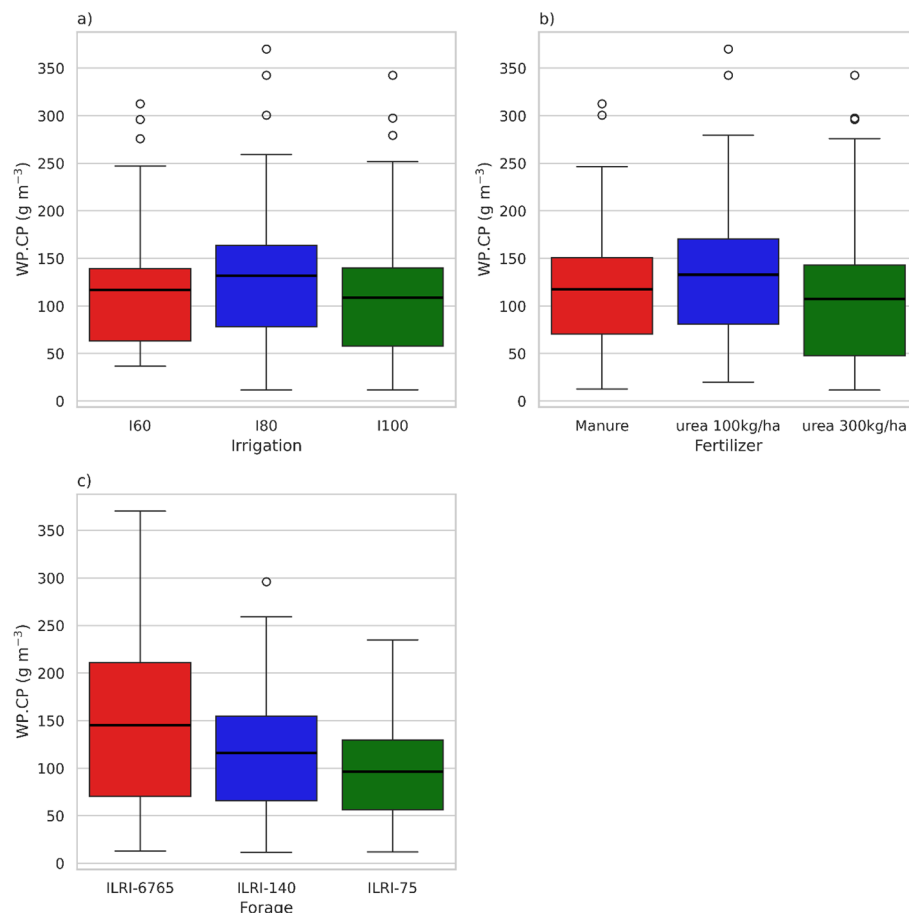


Fig. 4 Effect of irrigation (**a**), fertilizer (**b**), and forage species (**c**) on crude protein-based water productivity of three forage legume species (*D. uncinatum* ILRI 6765, *S. scabra* ILRI 140, and *S. hamata* ILRI 75) under three irrigation levels (I60, I80, I100) and fertilizer treatments in Robit watershed, Lake Tana basin, Ethiopia. Bars represent the mean $\pm$ standard error

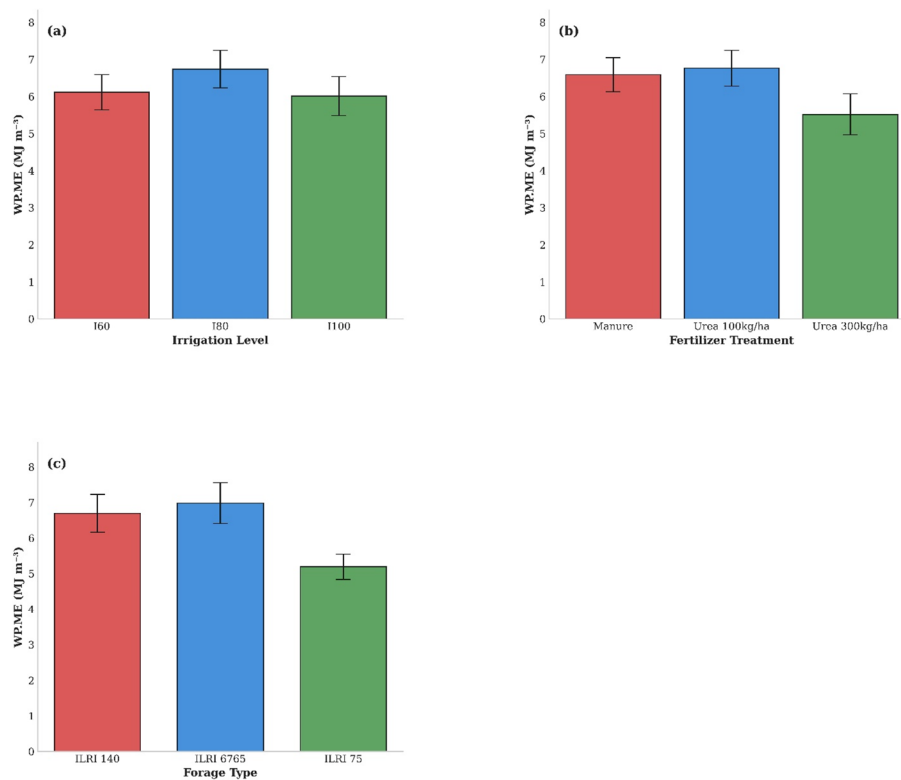


Fig. 5 Effect of irrigation (a), fertilizer (b), and forage species (c) on metabolizable energy-based water productivity of three forage legume species (*D. uncinatum* ILRI 6765, *S. scabra* ILRI 140, and *S. hamata* ILRI 75) under three irrigation levels (I60, I80, I100) and fertilizer treatments in Robit watershed, Lake Tana basin, Ethiopia. Bars represent the mean $\pm$ standard error

systems [19]. Under deficit irrigation (I60), limited soil water availability restricts cell expansion and stomatal conductance, thereby reducing photosynthetic carbon assimilation and forage accumulation production [20]; in contrast, full irrigation (I100) provides the water requirement to sustain near-maximum growth rates. Similar trends have been observed in other forage legume systems where irrigation improved both forage accumulation and resource use efficiency [21].

However, the response to fertilizer in this legume-based system differed markedly from our prior grass-based study at the same site Hussein et al., [5], where forage accumulation increased in a linear fashion with increasing urea rates. In contrast, in this legume experiment the 100 kg ha⁻¹ urea treatment gave the highest forage accumulation, while the highest urea rate (300 kg ha⁻¹) resulted in a lower forage accumulation than both the moderate urea and manure treatments. The absence of a zero-fertilizer control should be considered when interpreting fertilizer effects, as the results reflect relative differences among application levels rather than absolute responses. However the result is in agreement with the findings of Kamran et al., [21] who found a decreasing Alfafa (*Medicago sativa* L.) forage accumulation when fertilizer level increased. This reversal can be explained by unique physiological and ecological features of legumes. Legumes engage in symbiotic nitrogen fixation via root nodules with rhizobia. When soil mineral nitrogen becomes abundant (through high urea supply), legume plants often down-regulate nodulation and nitrogen fixation, shifting to uptake of available soil N [22]. This shift occurs at the cost of the prior energy investment in root nodules and

can reduce overall growth efficiency due to the metabolic burden of maintaining inactive nodules [23]. The forage accumulation response of the three tropical forage legumes to irrigation and nutrient supply reflects their unique physiology: strong reliance on water availability, moderated response to external nitrogen, and species-specific growth potential. These findings differ from grass-based systems and underscore the importance of designing forage management strategies specifically suited to legume biology.

The significant effect of forage species on forage accumulation is as a result of their inherent biological traits. *D.uncinatum* and *S.scabra* produced more forage accumulation than *S.hamata*, likely due to fundamental differences in their root systems, ability to form nitrogen-fixing nodules, tolerance to drought, and overall growth efficiency. Furthermore, tropical forage legumes are known to vary widely in their forage accumulation production and nitrogen fixation rates, with some species like *D.uncinatum* being particularly vigorous, while others, like certain *Stylosanthes* species, can be more sensitive to environmental stresses such as low soil fertility or moisture deficit. In general the forage accumulation observed in this study for the tropical forage legumes is generally less than that of forage grasses reported by Hussein et al., [5]. This difference reflects fundamental physiological differences between the two groups. Grasses typically achieve higher biomass due to faster growth rates and stronger responses to external nitrogen, whereas legumes allocate part of their assimilated carbon to symbiotic nitrogen fixation, which can limit aboveground biomass production [24]. Additionally, legumes are often more sensitive to environmental stresses, further constraining forage accumulation compared to grasses [25].

4.2 Water use efficiency

Water-use efficiency (WUE) in the current experiment was largely governed by forage species rather than by management of irrigation or fertilizer. The absence of a strong irrigation effect fits well with the concept that WUE can remain relatively stable across moderate soil moisture variations, because plants may sustain transpiration and assimilation until stress becomes severe [26]. In this trial, the three irrigation levels presumably maintained soil moisture within a functional range that allowed the legumes to operate without major reductions in efficiency, which may explain the non-significant differences in WUE. Likewise, fertilizer treatments did not significantly change WUE, suggesting that adding mineral nitrogen did not proportionally increase water-limited forage accumulation production. This result is consistent with the idea that, for legumes, nitrogen supply may become non-limiting due to biological fixation, so extra fertilizer does not necessarily boost forage accumulation per unit input [27]. The small decline in WUE under the highest urea rate likely reflects diminishing returns: when mineral N is overly abundant, growth may shift toward vegetative expansion at the expense of more water-efficient allocation, reducing the ratio of forage accumulation to water used [28].

In contrast to the limited effects of irrigation and fertilizer, species differences in WUE were significant. *D. uncinatum* ILRI 6765 and *S. scabra* ILRI 140 achieved significantly higher WUE than *S. hamata* ILRI 75, a pattern that likely stems from inherent physiological traits such as high transpiration efficiency, efficient canopy development, and strong root water uptake capacity.

The contrast between these findings and an earlier work on forage grasses Hussein et al., [5] is evident. In the grass study, WUE was highest under low irrigation (I60) and

increased with high nitrogen input, but in the legumes, the pattern was different. Grasses typically respond more strongly to mineral nitrogen, showing greater forage accumulation gains per unit water when additional N is available [27], while legumes rely more on their nitrogen-fixing symbiosis and may not benefit as much from very high urea inputs [28]. Furthermore, some grasses reduce stomatal conductance and maintain carbon gain under mild water stress, a strategy that may maintain forage accumulation and promote higher WUE under deficit irrigation; legumes, on the other hand, may continue high transpiration until stress is more intense.

From a practical standpoint, the results highlight that species choice is the primary determinant of water-use efficiency in tropical forage legumes, while moderate variations in irrigation or nitrogen supply offer limited gains. Selecting species with inherently high WUE, such as *D. uncinatum* ILRI 6765 or *S. scabra* ILRI 140, is therefore a more effective strategy to maximize water productivity under water-limited conditions. Careful nitrogen management is also important, as excessive fertilizer can suppress biological nitrogen fixation or alter forage biomass allocation, reducing efficiency [28]. These findings emphasize the value of integrating genotypic selection into forage management to enhance water productivity.

4.3 Nutritive value parameters

The lack of a statistically significant irrigation effect on key parameters like CP, NDF, and IVDOM indicates that water availability, within the tested range, acted more as a regulator of forage accumulation quantity than of its chemical composition. The slight increase in CP and IVDOM under the I80 regime is physiologically consistent with a non-linear response to water. Under moderate water availability (I80), plants can maintain optimal metabolic function and nitrogen assimilation without the potential for dilution of nutrients that can occur with excessive vegetative growth under very high water supply (I100) [29]. Similarly, water stress (I60) can limit metabolic processes, including protein synthesis.

The non-significant effect of fertilizer source and rate on forage nutritive value further underscores the nutritional self-sufficiency of these legumes. The slightly higher CP with 100 kg ha⁻¹ urea, followed by a decline at the higher 300 kg ha⁻¹ rate, aligns with the established concept of a nitrogen saturation point in fixating species. Moderate nitrogen may provide a readily available N source that supplements fixation, but higher doses can lead to feedback inhibition of nitrogenase activity, reducing the plant's own nitrogen fixation contribution and potentially disrupting the carbon-nitrogen balance necessary for optimal growth [30]. The intermediate nutritive value values from manure are consistent with its slow-release nature, providing a more gradual, but ultimately non-transformative, nutrient supply.

The strong and consistent significant differences among forage species for every nutritive value parameter demonstrates that genetic constitution is the dominant factor controlling nutritive value. The superior CP, ME and IVDOM of *D. uncinatum* ILRI 6765 likely stem from its inherent leaf-to-stem ratio and its ability to maintain a less lignified cell wall structure (as inferred from its lower ADL).

4.4 Nutrition and energy yield

The significant response of CP and ME yield to irrigation confirms that water availability was a key driver of forage accumulation. However, the lack of a significant increase in RFV yield reveals a critical limitation. While more water produced a larger volume of forage, it did not consistently improve its intrinsic nutritional concentration or digestibility. This is likely because water stress and sufficiency influence different plant processes. Ample water primarily promotes cell expansion and canopy growth, but it does not necessarily alter the fundamental biochemical pathways, such as lignification, that determine cell wall digestibility and ultimately define RFV. Therefore, irrigation boosts the quantity of harvestable nutrients, but the underlying nutritive value remains more tightly linked to genetic and maturity factors [31].

The consistent lack of a significant fertilizer effect demonstrates that in these legumes, nitrogen was not the primary forage accumulation-limiting factor. The plants' symbiotic relationship with rhizobia provided sufficient nitrogen to meet their growth demands under the experimental conditions. Adding external nitrogen, whether organic or mineral, provided no marginal benefit to nutrient forage accumulations. This is a classic observation in legume agronomy; once nitrogen fixation is established, additional fertilizer N often leads to diminishing returns in forage accumulations and can even disrupt the plant-rhizobia symbiosis, leading to a reduction in nitrogen fixation activity [32, 33].

The significant genotypic differences in nutrient yield (CP, ME, RFV) highlight that superior forage production depends on a species ability to synergistically combine high forage accumulation with high nutritive value. *D. uncinatum* ILRI 6765's leading performance suggests it possesses a genetic predisposition for efficient resource partitioning; directing assimilates towards the production of digestible, protein-rich leaves rather than lignified stems. This genetic control over the critical balance between forage accumulation and nutritive value is a well-documented lever for improving forage systems [34].

Overall, improving the nutritional and energy yield of these forage legumes relies mainly on choosing the right varieties, as genetics has the strongest influence. Irrigation can then be adjusted to help these forages reach their potential, though its main benefit is increasing forage accumulation. Since nitrogen fertilizers had little effect, the results highlight the value of biological nitrogen fixation and suggest that nutrient management should focus on efficiency rather than heavy inputs.

4.5 Nutrient based water use efficiency

The absence of a significant irrigation effect on water productivity in terms of crude protein and metabolizable energy reveals, while increased water application boosted total forage accumulation and nutrient yield, it did not enhance the efficiency with which water was converted into nutritional value. This suggests a trade-off where the additional water primarily supported structural growth and maintenance, rather than proportionally increasing the synthesis of digestible nutrients. The result is that moderate irrigation (I80) can achieve forage accumulations comparable to full irrigation (I100) with improved nutrient-based water productivity. This pattern is consistent with studies on other forage legumes, where deficit irrigation strategies often achieve WUE values equivalent to or even exceeding those of full irrigation, as plants under mild stress can maintain photosynthetic rates while reducing water loss [35]. This indicates that optimal

irrigation scheduling should target maximum nutrient forage accumulation per unit of water, not merely maximum forage accumulation.

The negligible impact of nitrogen fertilizer on all metrics, including WP.CP and WP.ME, strongly indicates that the legumes in this study were not primarily nitrogen-limited. As nitrogen-fixing species, they likely met their nitrogen demand through symbiotic atmospheric fixation, rendering the supplemental applications whether organic or mineral not that much necessary. This efficient self-sufficiency in nitrogen acquisition is a key feature of legumes. The result reinforces the concept that in such systems, the capacity for biological N₂ fixation and internal nitrogen remobilization is a more powerful governor of protein production than soil nitrogen availability [36]. Consequently, management efforts might be more effectively directed toward optimizing factors that support effective nodulation and N-fixation, such as phosphorus availability and soil pH, rather than applying direct nitrogen fertilizers, which can sometimes even inhibit the symbiotic process [37].

The pronounced differences in water productivity for crude protein (WP.CP) and Metabolizable energy (WP.ME) among the forage species underscore that intrinsic physiological traits are the primary drivers of nutrient production per unit of water. The superior performance of *D. uncinatum* ILRI 6765 reflects a synergistic combination of high forage accumulation and a favourable partitioning of resources into protein and energy-rich leaf mass, rather than structural stem material. This efficient resource partitioning is a key determinant of forage nutritive value. Our findings align with established principles that genotypic variation in canopy architecture, photosynthetic capacity, and carbon allocation patterns are major sources of variation in water-use efficiency for forage accumulation production in crops and forages. Therefore, selecting species with these inherent efficiency traits offers a fundamental strategy for enhancing sustainable forage production in water-limited environments.

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5 Conclusions

This study shows that species selection is the dominant factor determining forage accumulation, water use efficiency, and nutritive value of tropical forage legumes in the sub-humid Ethiopian highlands. *D. uncinatum* ILRI 6765 and *S. scabra* ILRI 140 consistently outperformed *S. hamata* ILRI 75 across forage accumulation and nutritive value indicators. Irrigation influenced forage accumulation, but moderate irrigation (I80) achieved a better balance between forage accumulation, water use efficiency, and forage nutritive value than full irrigation. In contrast, nitrogen application had limited effects, indicating that the forage legumes can maintain performance with minimal external nitrogen inputs.

These findings highlight that improving forage performance under water-limited conditions is best achieved through careful species selection rather than increased irrigation or fertilizer use. Prioritizing well-adapted, efficient legume species such as *D. uncinatum* offers a practical and resource-efficient pathway to enhance forage accumulation and nutritional value in smallholder systems. Future research should build on this evidence by evaluating how such high-performing legumes can be integrated with complementary forage species, including grasses, to improve system-level forage accumulation, water-use efficiency, and long-term sustainability under variable water and soil fertility conditions.

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Author contributions

Misbah A. Hussein: Writing – original draft, Methodology, Data curation, Investigation, Conceptualization, Formal analysis. Chalie W. Taye: Data curation, Writing – review & editing. Fikadu T. Riga: Writing – review & editing, Resources, Data curation. Tewodros T. Assefa: Writing – review & editing, Conceptualization, Methodology. Melkamu B. Derseh: Writing – review & editing, Project administration, Funding acquisition, Conceptualization. Seifu A. Tilahun: Writing – review & editing, Methodology, Conceptualization.

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Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests

The authors declare no competing interests.

Field data collection permission

Field experiments were conducted on privately leased farmland in Robit Bata Kebele, Bahir Dar Zuria Woreda, Ethiopia. The land was used under a formal lease agreement with the landowner, which granted permission for forage cultivation, management, harvesting, and data collection. All activities complied with local regulations.

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References

1. Rai S. Improved Agronomic Practices on Enhancement of Quality Forage Production for Livestock Farming. *Agron J Nepal*. 2024;8:226–36. <https://doi.org/10.3126/ajn.v8i1.70917>.
2. Pastorelli G, Serra V, Vannuccini C, Attard E. *Opuntia* spp. as Alternative Fodder for Sustainable Livestock Production. *Animals*. 2022;12:1597. <https://doi.org/10.3390/ani12131597>.
3. Tulu D, Gadissa S, Hundessa F, Kebede E. Contribution of Climate-Smart Forage and Fodder Production for Sustainable Livestock Production and Environment: Lessons and Challenges from Ethiopia. *Adv Agric*. 2023;2023:8067776. <https://doi.org/10.1155/2023/8067776>.
4. De Haan K, Khomik M, Green A, et al. Assessment of Different Water Use Efficiency Calculations for Dominant Forage Crops in the Great Lakes Basin. *Agriculture*. 2021;11:739. <https://doi.org/10.3390/agriculture11080739>.
5. Hussein MA, Hailelassie A, Derseh MB, et al. Enhancing irrigated forage crop production through water and nutrient management in the Ethiopian sub-humid highlands. *Front Sustain Food Syst*. 2024;8. <https://doi.org/10.3389/fsufs.2024.1373698>.
6. Hussein MA, Riga FT, Derseh MB, et al. Application of Irrigation Management and Water-Lifting Technologies to Enhance Fodder Productivity in Smallholder Farming Communities: A Case Study in Robit Bata, Ethiopia. *Agronomy*. 2024;14:1064. <https://doi.org/10.3390/agronomy14051064>.
7. Kumar R, Yadav MR, Arif M, et al. Multiple agroecosystem services of forage legumes towards agriculture sustainability: An overview. *Indian J Agric Sci*. 2020;90:1367–77. <https://doi.org/10.56093/ijas.v90i8.105882>.
8. Mahmud K, Makaju S, Ibrahim R, Missaoui A. Current Progress in Nitrogen Fixing Plants and Microbiome Research. *Plants*. 2020;9:97. <https://doi.org/10.3390/plants9010097>.
9. Taye CW, Hussein MA, Ayalew AD, et al. Evaluating irrigation and fertilizer strategies for sustainable forage productivity and improved nitrogen efficiency in the sub-humid highlands of Ethiopia. *J Agric Food Res*. 2025;23:102242. <https://doi.org/10.1016/j.jafr.2025.102242>.
10. Worqlul AW, Dile YT, Schmitter P, et al. Constraints of small-scale irrigated fodder production and nutrition assessment for livestock feed, a case study in Ethiopia. *Agric Water Manag*. 2021;254:106973. <https://doi.org/10.1016/j.agwat.2021.106973>.
11. Amar AL, Congdon R, Coventry R, Gardiner C. Responses of selected tropical forage legumes to imposed drought. *Sci Arch*. 2022;3:158–67.
12. Lagrange SP, MacAdam JW, Villalba JJ. The Use of Temperate Tannin Containing Forage Legumes to Improve Sustainability in Forage–Livestock Production. *Agronomy*. 2021;11:2264. <https://doi.org/10.3390/agronomy11112264>.
13. Lee MA. A global comparison of the nutritive values of forage plants grown in contrasting environments. *J Plant Res*. 2018;131:641–54. <https://doi.org/10.1007/s10265-018-1024-y>.
14. Fenta HM, Hussein MA, Tilahun SA, et al. *Berken* plow and intercropping with pigeon pea ameliorate degraded soils with a hardpan in the Ethiopian highlands. *Geoderma*. 2022;407:115523. <https://doi.org/10.1016/j.geoderma.2021.115523>.
15. Dadrasan M, Chaichi MR, Pourbabaee AA, et al. Deficit irrigation and biological fertilizer influence on yield and trigonelline production of fenugreek. *Ind Crops Prod*. 2015;77:156–62. <https://doi.org/10.1016/j.indcrop.2015.08.040>.

16. Rostamza M, Chaichi M-R, Jahansou M-R, Alimadadi A. Forage quality, water use and nitrogen utilization efficiencies of pearl millet (*Pennisetum americanum* L.) grown under different soil moisture and nitrogen levels. *Agric Water Manag.* 2011;98:1607–14. <https://doi.org/10.1016/j.agwat.2011.05.014>.
17. Sime G, Aune JB. Maize Response to Fertilizer Dosing at Three Sites in the Central Rift Valley of Ethiopia. *Agronomy.* 2014;4:436–51. <https://doi.org/10.3390/agronomy4030436>.
18. Rohweder DA, Barnes RF, Jorgensen N. Proposed Hay Grading Standards Based on Laboratory Analyses for Evaluating Quality. *J Anim Sci.* 1978;47:747–59. <https://doi.org/10.2527/jas1978.473747x>.
19. Aviles DF, Cruz A, Cooper CE, et al. Assessment of Alternative Warm-Season Annual Grasses for Forage Production in Water-Limited Environments. *Grasses.* 2025;4:36. <https://doi.org/10.3390/grasses4030036>.
20. Melo AS de, Silva ARF da, Dutra AF, et al. Photosynthetic efficiency and production of cowpea cultivars under deficit irrigation. *Rev Ambiente Água.* 2018;13(e2133). <https://doi.org/10.4136/ambi-agua.2133>.
21. Kamran M, Yan Z, Jia Q, et al. Irrigation and nitrogen fertilization influence on alfalfa yield, nutritive value, and resource use efficiency in an arid environment. *Field Crops Res.* 2022;284:108587. <https://doi.org/10.1016/j.fcr.2022.108587>.
22. Streeter J, Wong PP. Inhibition of legume nodule formation and N₂ fixation by nitrate. *Crit Rev Plant Sci.* 1988. <https://doi.org/10.1080/07352688809382257>.
23. Vance CP. Symbiotic Nitrogen Fixation and Phosphorus Acquisition. *Plant Nutrition in a World of Declining Renewable Resources.* *Plant Physiol.* 2001;127:390–7. <https://doi.org/10.1104/pp.010331>.
24. Lüscher A, Mueller-Harvey I, Soussana JF, et al. Potential of legume-based grassland–livestock systems in Europe: a review. *Grass Forage Sci.* 2014;69:206–28. <https://doi.org/10.1111/gfs.12124>.
25. Soussana J-F, Lüscher A. Temperate grasslands and global atmospheric change: a review. *Grass Forage Sci.* 2007;62:127–34. <https://doi.org/10.1111/j.1365-2494.2007.00577.x>.
26. Blum A. Effective use of water (EUW) and not water-use efficiency (WUE) is the target of crop yield improvement under drought stress. *Field Crops Res.* 2009;112:119–23. <https://doi.org/10.1016/j.fcr.2009.03.009>.
27. Sinclair TR, Rufty TW. Nitrogen and water resources commonly limit crop yield increases, not necessarily plant genetics. *Glob Food Secur.* 2012;1:94–8. <https://doi.org/10.1016/j.gfs.2012.07.001>.
28. Reinprecht Y, Schram L, Marsolais F, et al. Effects of Nitrogen Application on Nitrogen Fixation in Common Bean Production. *Front Plant Sci.* 2020;11:1172. <https://doi.org/10.3389/fpls.2020.01172>.
29. Parlitz S, Kunze R, Mueller-Roeber B, Balazadeh S. Regulation of photosynthesis and transcription factor expression by leaf shading and re-illumination in *Arabidopsis thaliana* leaves. *J Plant Physiol.* 2011;168:1311–9. <https://doi.org/10.1016/j.jplph.2011.02.001>.
30. Voisin AS, Salon C, Jeudy C, Warembourg FR. Symbiotic N₂ fixation activity in relation to C economy of *Pisum sativum* L. as a function of plant phenology. *J Exp Bot.* 2003;54:2733–44. <https://doi.org/10.1093/jxb/erg290>.
31. Hornick SB. Nutritional Quality of Crops as Affected by Management Practices.
32. Pampana S, Masoni A, Mariotti M, et al. Nitrogen fixation of grain legumes differs in response to nitrogen fertilisation. *Exp Agric.* 2018;54:66–82. <https://doi.org/10.1017/S0014479716000685>.
33. Sangakkara UR, Marambe B. Effect of Method of Inoculation and Nitrogen Fertilizer on Nodulation and Yield of Selected Tropical Legumes. *J Agron Crop Sci.* 1989;162:305–9. <https://doi.org/10.1111/j.1439-037X.1989.tb00721.x>.
34. Alexander G, Ravi D, Ramakrishna Reddy C, et al. Forage yield and quality in pigeonpea germplasm lines. *J SAT Agric Res.* 2007;3:1–3.
35. López-Olivari R, Ortega-Klose F. Response of red clover to deficit irrigation: dry matter yield, populations, and irrigation water use efficiency in southern Chile. *Irrig Sci.* 2021;39:173–89. <https://doi.org/10.1007/s00271-020-00693-0>.
36. Locke AM, Ramirez ME. Increased nitrogen fixation and remobilization may contribute to higher seed protein without a yield penalty in a soybean introgression line. *bioRxiv.* 2020;602748. <https://doi.org/10.1101/602748>.
37. Li Q, Philp J, Denton MD, et al. Stoichiometric homeostasis of N:P ratio drives species-specific symbiotic N fixation inhibition under N addition. *Front Plant Sci.* 2023;14. <https://doi.org/10.3389/fpls.2023.1076894>.

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