

Article

Measuring the Influence of Key Management Decisions on the Nitrogen Nutritional Status of Annual Ryegrass-Based Forage Crops

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Abstract: Increasing nitrogen use efficiency (NUE) by improving agricultural practices and soil knowledge, and implementing precision agriculture, is essential to reduce the overuse of fertilizers and increase nutrient retention. This study aimed to optimize N management in agriculture by establishing a critical N dilution curve (CNDC) and analyzing variations in NUE and the N nutrition index (NNI) among different crops under various treatments. Using a Bayesian model, the CNDC was determined as $\%N_c = 3.63 \times PDM^{-0.71}$. The results showed that plant dry matter (PDM) and plant N content (PNC) varied significantly with crop type and sampling moments. Strong positive correlations are presented by PDM with N uptake (NUp) (0.89) and NNI (0.88), along with an inverse correlation with critical N concentration (−0.95). The study found that crops under irrigation conditions had higher NUp and higher NNI. This study provides valuable insights into the influence of key management decisions on the N nutritional status of annual ryegrass-based forage crops. The results highlight the critical role of accurate and conscious decision-making in improving NUE and crop yields, emphasizing the complex interactions between biomass production and N dynamics in crops. The conclusions allow significant benefits to be realized, contributing to the sustainability of agricultural systems.

Keywords: critical nitrogen concentration; data-driven approach; crop yield; site-specific analysis; sustainability; variable rate fertilization



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1. Introduction

The instability and high level of fertilizer prices is a problem. Fertilizer costs represent for farmers in the European Union, on average, around 6% of input costs and can reach up to 12% for arable crop producers [1]. Increasing nitrogen use efficiency (NUE) by improving agricultural practices and soil knowledge, and implementing precision agriculture, as well as strengthening pollution prevention through nitrate action programs, are essential to reduce the overuse of fertilizers and increase nutrient retention. These efficiency gains, as well as leading to clear benefits, will reduce tensions in the world market [1,2]. Adapting agronomic practices is essential and has the potential to stabilize or increase yields, especially in Mediterranean areas, in response to the specific regional constraints [3].

Diagnosing a crop's N nutritional status based on the plant's N concentration relies on the concept of critical N concentration ($\%N_c$), which is defined by the N concentration required to achieve maximum vegetative growth [4]. The dilution of N in plant tissues during crop growth has been widely discussed by various authors, who have applied different mathematical models to describe it. Initially, Lemaire et al. [5] established the concept of the critical N dilution curve (CNDC). This allometric approach describes the decline in $\%N_c$ as the accumulation of plant dry matter (PDM; $t\ ha^{-1}$). By measuring $\%N_c$, it is possible to determine its N nutrition index (NNI), quantifying the intensity of the deficit or excess consumption of the macronutrient [6]. For a given amount of PDM, NNI can be estimated by comparing the current plant N concentration (PNC; %), with $\%N_c$ calculated by the CNDC [7]. An NNI value equal to or close to 1 means the crop is at the optimum value to achieve maximum yield. This value can be used as a yield and quality reference to correct gaps in crop management [8]. It should be noted that the NNI can only diagnose the N status of the crop but cannot quantify the dose of N to be applied in top dressing, especially when the NNI is less than 1.

Makowski et al. [9] were concerned that these CNDC values (sequential method) did not have the level of uncertainty with which they predict the $\%N_c$, and recommended the Bayesian model (hierarchical method) to estimate the parameters of the CNDC from experimental data in a single step. Using freely available software, the model can estimate the curve parameters directly from the PDM-PNC data pair. Lacasa et al. [10] compared both statistical methods and evaluated their performance, considering different observation times throughout the crop cycle, different fertilizer doses, and different levels of measurement accuracy. They showed that the sequential models result in poor statistical properties of the estimated parameters and overestimate the $\%N_c$ values. The estimates of N requirements to reach N sufficiency levels exceeded the values estimated by the hierarchical model by around 30 to 100 kg N ha^{-1} . Lacasa et al. [10] corroborated the model presented by Makowski et al. [9], indicating that its use reduces the risk of over-fertilization.

In addition, losses of N into the environment have become a major concern. The knowledge of the NUE and its components is important to determine crop N requirements and to define N fertilization strategies that reduce N losses and, consequently, the risks of environmental contamination [11]. Variations in N use parameters could be expected for the same value of the NNI, because of different PDM values in response to genotype (G) \times management (M) \times environmental (E) conditions. Quantitatively assessing N use parameters in response to the N nutrition index will enhance our understanding of how these parameters vary under different G \times M \times E conditions. This is achieved by isolating the effects of nutritional status and biomass on these parameters. Additionally, this approach will help define a reference value for N use parameters under optimal N nutritional status. This reference value can be used to benchmark N management practices aimed at improving NUE in sustainable agricultural systems [12].

The objectives of this study were (1) to determine the best fit of the data to the CNDC (critical N dilution curve) and NNI (NUE and N nutrition index) using a Bayesian method, and (2) to differentiate ryegrass-based cropping systems, N treatments, water management strategies, and growth stages.

2. Materials and Methods

2.1. Field Experiments

The experimental trials were conducted in Herdade da Comenda—Innovation Centre of the National Institute for Agricultural and Veterinary Research (INIAV), in Caia, Elvas, Portugal (38°53'38.52" N and 7°3'19.01" W) between 28 November 2022 and 14 April 2023 (137 days). The trial design followed a split-plot randomized block model [13,14]. The main block had 3 doses of N topdressing (N0, N1, and N2 with 0, 120, and 200 kg ha^{-1} of fertilizer with 27% N, respectively). The sub-blocks had two different forage crops, and the sub-sub-blocks had two irrigation management scenarios (rainfed vs. irrigated). In irrigated management, water was applied at key times in the crop (sowing, ryegrass

tillering, and ryegrass stem elongation) until the soil reached field capacity. The plots in the blocks had a total size of 2×8 m (Figure 1). Each plot was replicated 4 times, totaling 48 plots. They were set up in a flat location to avoid the potential influence of runoff of water or nutrients.

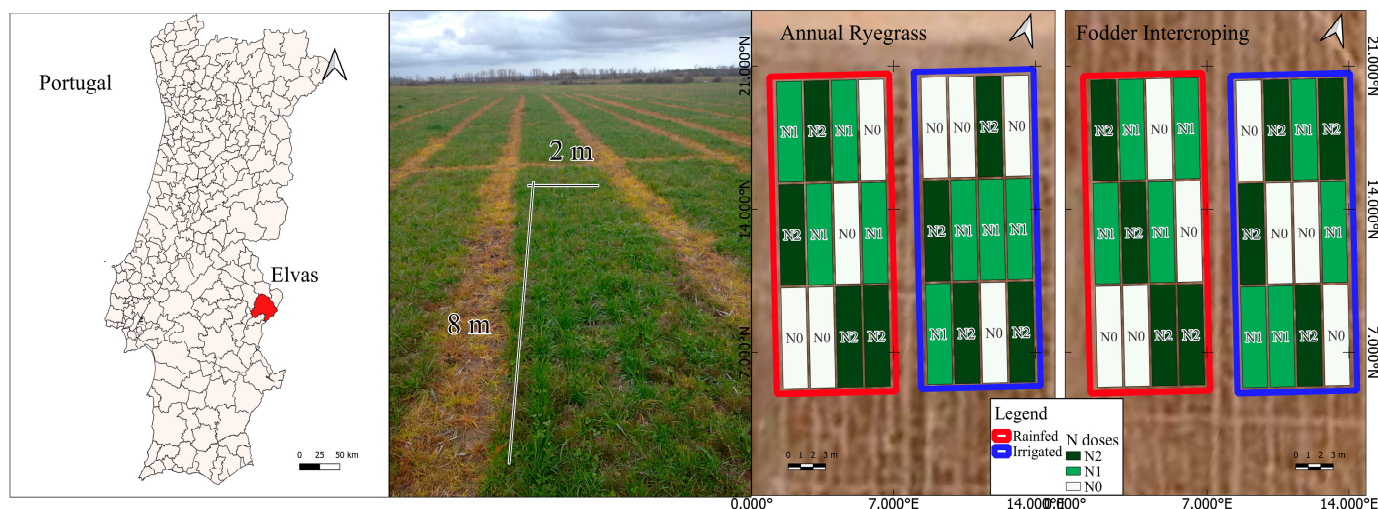


Figure 1. Location and design of the experiment field.

The crops of (i) annual ryegrass and (ii) forage intercropping based on annual ryegrass and clovers (Table 1) were sown separately with an inter-row of 0.18 m and a depth of 0.01 m. The sowing doses were $1400 \text{ seeds m}^{-2}$ for annual ryegrass and $4070 \text{ seeds m}^{-2}$ for intercropping [15], seeded on 7 November 2022. Before sowing, on 28 October 2022, sufficient doses of phosphorus ($16.1 \text{ kg ha}^{-1} \text{ P}_2\text{O}_5$) and potassium ($13.8 \text{ kg ha}^{-1} \text{ K}_2\text{O}$) were applied, along with a dose of N ($27.6 \text{ kg ha}^{-1} \text{ N}$) distributed evenly across all the plots to ensure correct crop emergence. Irrigation was also applied evenly at this time, using a sprinkler system. This irrigation was carried out until the soil reached field capacity and allowed the crop to emerge. Weeds and diseases were controlled following good experimental practices.

Table 1. Main characteristics of the ryegrass-based forage crops used.

Crop	Species and Varieties	Percent in the Crop (%)	Seeds m^{-2}
Annual ryegrass (RG)	<i>Lolium multiflorum</i> L. cv Diamond T	100	1400
Intercropping ryegrass-based (Int)	<i>Lolium multiflorum</i> L. cv Hellen	67	1531
	<i>Trifolium vesiculosum</i> . cv Comm	10	
	<i>Trifolium resupinatum</i> . cv Lightning	17	2539 ¹
	<i>Trifolium michellanium</i> . cv Balansa Paradana	6	

¹ The value present represents the sum of the number of seeds of the three clover varieties.

The predominant soil at the test site, according to the FAO classification, is a Luvisol, which corresponds to Mediterranean soils Pag and Sr [16], and the climate, according to Köppen–Geiger, is Csa [17]. The average monthly temperature (T_m ; °C) and rainfall (P ; mm) for the climatological normal (1971–2000) [18] and the test period are shown in Table 2.

At two stages in the crop cycle (ryegrass tillering— t_1 ; ryegrass stem elongation— t_2), plants were cut 5 cm above the ground in a sub-sampling area ($1 \text{ m} \times 1 \text{ m}$) at the northernmost point of each plot. In total, 98 samples were collected. These samples included all vegetation above the cutting height within the area, allowing for the estimation of plant fresh matter. The samples were dried in an oven at $60 \text{ }^\circ\text{C}$ until they reached a constant dry weight (approximately 24–48 h). After drying, the samples were weighed to

determine the PDM. They were then ground and analyzed using the Kjeldahl method [19] to estimate the PNC. This process collected the PDM-PNC data pair for each plot.

Table 2. Average monthly temperature (T_m ; °C) and rainfall (P; mm) for the climatological normal (1971–2000) and the test period.

Month	T_m Normal (°C)	T_m (°C)	P Normal (mm)	P (mm)
October	17.4	19.2 ¹	58.6	14.3 ¹
November	12.5	14.1	75.1	37.4
December	9.7	12.8	92.6	277.7
January	8.6	8.9	63.1	40.0
February	10.2	10.1	54.6	4.9
March	12.3	13.7	39.6	18.5
April	14.1	16.2 ²	51.2	0.0 ²

¹ only the last two days of the month; ² only the first fourteen days of the month. T_m —average daily temperature (°C); P—precipitation (mm).

2.2. Fit CNDC

The R code created by Makowski et al. [9] was used to adjust the CNDC, in which the PDM-PNC data pairs from each experiment plot were entered. CNDC settings were developed in RStudio©v.2023.4.3.2 with the R©jags Package v. 4-15 CRAN Repository [20,21]. With this adjustment, the values of the A1 and A2 parameters of the CNDC (Equation (1)) were estimated using the most appropriate curve for estimating the %N_c of these crops, installed by no-till in sandy soil, in this particular year.

$$\%N_c = A1 \times PDM^{-A2}, \quad (1)$$

where %N_c is the total concentration of N in the forage (which produces an amount of plant dry matter (PDM; t ha⁻¹), expressed in g N kg⁻¹ of forage PDM; A1 is the coefficient that represents the %N_c of the forage at 1 t PDM ha⁻¹; and A2 is the coefficient that characterizes the pattern of decrease in %N_c during crop growth [22].

Since the model for calculating the CNDC should not incorporate data pairs with PDM of less than 1 t ha⁻¹, of the 96 initial pairs, only 58 were considered for adjusting the N curve. The %N_c value was calculated based on the PNC of annual ryegrass and intercropping together, because, given the similarities in the crops, other authors have already proven that the values of the CNDC parameters do not change from one crop to another [23,24]. The curves achieved by other authors in fodder crops, already described in the introduction and specified in Table 3, were considered.

Table 3. Prior information was used on A1 and A2 coefficients for fodder crops.

Crop/Species	A1	A2	Ref.
<i>Festuca arundinacea</i> Schreb. and <i>Dactylis glomerata</i> L.	4.8	0.32	[5]
C3 crops	5.7	0.50	[25]
<i>Lolium multiflorum</i> L. ¹	4.1	0.38	[11]
<i>Pheleum pratense</i> L.	3.7	0.35	[26]
<i>Lolium perenne</i> L.	6.3	0.71	[27]
<i>Lolium multiflorum</i> L. ²	3.5	0.36	[28]
<i>Festuca arundinacea</i> Schreb.	4.7	0.55	[28]
<i>Avena sativa</i> L.	3.2	0.26	[28]
Minimum	3.2	0.26	
Maximum	6.3	0.71	

¹ rainfed annual ryegrass; ² irrigated annual ryegrass.

Following the Lemaire et al. [5] CNDC validated for fescue, Greenwood et al. [25] proposed a simplification by creating a general curve for C3 crops and another for C4 crops. However, other authors created and validated CNDCs specifically for forage crops such as

rained annual ryegrass [11], timothy grass [26], perennial ryegrass [27], irrigated annual ryegrass, oats, and again for fescue [28], and hybrid-annual ryegrass [6]. These curves differ; the value of A1 varies between 3.2 and 6.3, and the value of A2 is between 0.26 and 0.71 (Table 1). These variations prove the consensus presented by most authors that these curves vary depending on the year (climatic conditions), the crop, the variety, and the crop management practiced.

2.3. N Use Parameters and NNI

NUE and its two parameters, N uptake (NUp; Equation (2)) and N uptake efficiency (NUpE; Equation (3)), were calculated according to the method presented by Marino et al. [11].

$$\text{NUp} = \text{PDM} \times \text{PNC}, \quad (2)$$

where NUp is the N uptake by the crop in kg ha^{-1} , expressing the amount of N absorbed in PDM per hectare. It is the result of multiplying PDM with PNC. Equation (3) presents the formula for calculating NUpE.

$$\text{NUpE} = [(\text{NUp fertilized plot} - \text{NUp control plot}) / \text{N applied}] \times 100, \quad (3)$$

where NUpE (N uptake efficiency) measures the efficiency of N utilization as a percentage. It is calculated as the difference in N absorption (NUp) between the fertilized plots and the control plots, divided by the amount of N applied as topdressing at ryegrass tillering (kg ha^{-1}). Finally, the calculation of NUE (N Use Efficiency) is presented in Equation (4):

$$\text{NUE} = (\text{PDM of N-fertilized plot} - \text{PDM of control plot}) / \text{N applied}, \quad (4)$$

where NUE (kg kg^{-1} N) represents the amount of PDM produced per kg of N applied as topdressing. It is calculated by dividing the difference between the PDM of the topdressing fertilized plot (kg ha^{-1}) and the PDM of the control plot (kg ha^{-1}) by the amount of N applied (kg ha^{-1}).

Knowing the value of PNC and $\%N_c$, it is possible to calculate the NNI using the ratio between these two parameters (Equation (5)):

$$\text{NNI} = \frac{\text{PNC}}{\%N_c}, \quad (5)$$

where PNC is the actual concentration of the N in plants and $\%N_c$ is the optimum concentration of N in the plant.

2.4. Statistical Analysis

A statistical and inferential analysis was carried out to begin analyzing the general data, including the coefficient of variation, standard deviation, and interquartile analysis, in RStudio©v.2023.4.3.2 with the R©psych Package v. 2.4.3 CRAN Repository [20,29]. The data were then grouped by crop, moment, N treatment, irrigation management, and the dependent variables (the parameters PDM, PNC, NUp, NUpE, $\%N_c$, and NNI).

The Shapiro–Wilk test was applied to each dependent variable as a normality test. Variables with a non-normal distribution were analyzed using the Kruskal–Wallis test and those with a normal distribution were analyzed using ANOVA, as presented in Figure 2.

The post hoc analyses that followed the Kruskal–Wallis test used Dunn’s test in RStudio©v.2023.4.3.2 with the R©dunn.test Package v. 1.3.6 CRAN Repository [20,30]. In the case of post hoc analyses following ANOVA, Tukey’s HSD test was used.

Finally, a correlation matrix was used to find strong relationships between the dependent variables.

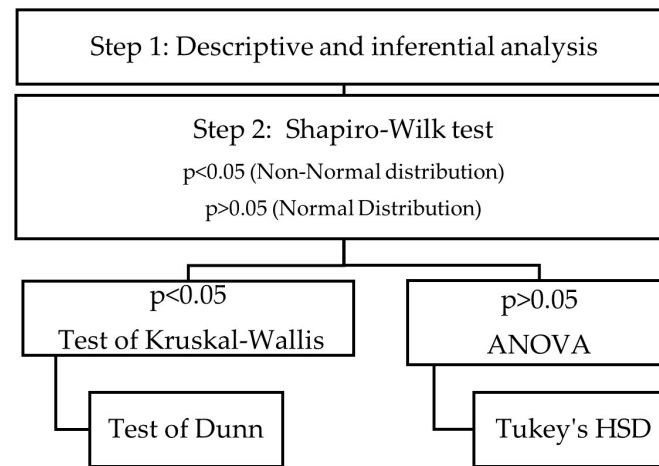


Figure 2. Diagram of the statistical data analysis procedure.

3. Results

3.1. Critical N Dilution Curve

The CNDC of the crops under study was established using the Bayesian statistical model, according to Makowski et al. [9]. The confidence interval (CI) associated with all the data (PDM and PNC) recorded was 97.5%. Samples with PDM less than 1 t ha⁻¹ were excluded from the curve fitting. The A1 and A2 parameters range from 3.18 to 3.87 and 0.53 to 0.72, respectively. Finally, the CNDC that was determined is %N_c = 3.63 × PDM^{-0.71} (Table 4).

Table 4. Estimated data for coefficients A1 and A2.

	CI 97.5%	Mean	SD	Naive SE	Time-Series SE
A1	3.634	3.356	0.119	0.00	0.00
A2	0.708	0.657	0.036	0.00	0.00

A1—%N_c of the forage at 1 t PDM ha⁻¹; A2—coefficient that characterizes the pattern of decrease in %N_c during crop growth; CI—confidence interval; SD—standard deviation; SE—standard error.

Figure 3 presents the distribution of the estimated A1 (a) and A2 (b) parameters.

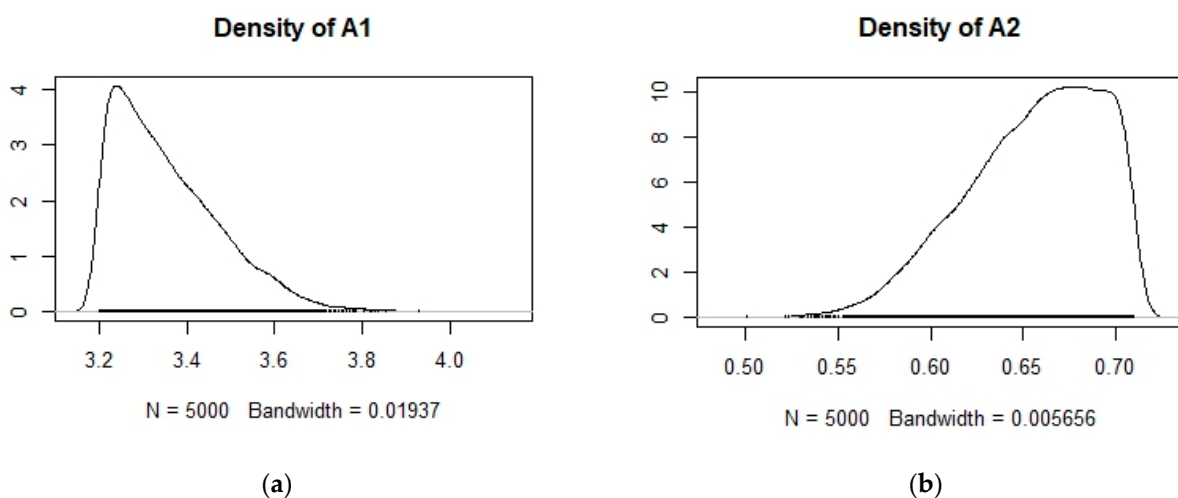


Figure 3. Estimated values at CI 97.5% of parameters A1 ((a) in %) and A2 ((b) in %).

3.2. Variations of Crops N Use Parameters and NNI

The total database has 96 pairs of PDM-PNC data, 48 of which are from moment t1 and 48 from moment t2. As NUP was calculated for moment t2, only 48 samples were

counted. In the case of NUpE and NUE, only 32 samples were counted because they were only calculated for moment t2, excluding the N0 samples. Therefore, the N use efficiency performance indicators were calculated at stem elongation for the topdressing N applied at moment t1, when the ryegrass was in the tillering phase. In the calculation process, each plot was used individually. The unique identification of each plot drawn at random at the beginning of the experimental design was used to calculate N use parameters.

After removing the outliers, the statistical analysis was carried out, and the cleaned data are presented in Table 5.

Table 5. Descriptive and inferential statistics were conducted for the parameters PDM, PNC, NUp, NUpE, NUE, %N_c, and NNI, excluding outliers.

	PDM (t ha ⁻¹)	PNC (%)	NUp (kg ha ⁻¹)	NUpE (%)	NUE (kg kg N ha ⁻¹)	%N _c (%)	NNI
N	96	95	45	32	32	60	58
mean	1.78	1.66	43.96	32.92	11.82	2.03	0.91
sd	1.15	0.28	14.63	52.87	26.97	0.56	0.28
median	1.44	1.64	44.60	35.52	10.29	1.89	0.87
trimmed	1.69	1.64	43.51	30.11	11.95	1.98	0.89
mad	1.15	0.32	14.46	54.74	22.75	0.61	0.30
min	0.34	1.15	17.92	−62.11	−45.69	1.15	0.48
max	5.02	2.29	73.29	161.99	67.53	3.27	1.63

n—number of samples; sd—standard deviation; mad—absolute deviation from median; min—minimum; max—maximum; PDM—plant dry matter; PNC—plant nitrogen content; NUp—nitrogen uptake; NUpE—nitrogen uptake efficiency; NUE—nitrogen use efficiency; %N_c—critical nitrogen concentration; NNI—nitrogen nutrition index.

3.2.1. Variables with Non-Normal Distribution

The data for the PDM, PNC, NUp, %N_c, and NNI variables do not follow a normal distribution, so the Kruskal–Wallis test was used as a non-parametric alternative to assess the influence of the Crop, Moment, Irrigation, and N treatment factors. The results of the Kruskal–Wallis test indicate that all these factors respond to the timing of the crop and also vary from crop to crop. Only NUp responds to the Irrigation factor. These results are presented in Table 6, and the boxplots of the factors that had significance are presented in Figure 4.

The results of the Kruskal–Wallis test indicate that there is a significant difference between the Crop groups for PDM and a highly significant difference between the Moment groups, also for PDM. Among the Irrigation groups, there is marginal significance for the same variable. There is no significant difference between the N treatment groups for PDM.

For PNC, there is a highly significant difference between the Crop groups and a significant difference between the Moment groups. There is no significant difference between the Irrigation and N treatment groups for PNC.

The NUp has a significant difference by Irrigation, and there is a marginal influence between Crop and N treatment groups. Moment was not considered for NUp because the data are only from t2. At t1, the application of N and water had not yet taken place.

The Moment groups present a highly significant difference for %N_c and NNI. The Irrigation groups present a marginal difference in both %N_c and NNI, the Crop groups only present a marginal difference in %N_c, and the N treatment groups present no significance in either %N_c or NNI.

For the N treatment group, which presented some significance for NUp, post hoc analyses were carried out to identify which groups differed from one another. Dunn's test was used for multiple comparisons. The results are presented in Table 7.

The NUp presents a highly significant difference between the water treatments, with an adjusted *p*-value of 0.0090 (*p* < 0.01). Comparisons between N0 and N1 are significant, as they present an adjusted *p*-value of 0.0363 (*p* < 0.05), while N0 vs. N2 and N1 vs. N2 present no significant differences.

Table 6. Results of the Kruskal–Wallis test for dependent variables that do not follow a normal distribution.

	Factor	Chi-Squared	df	p-Value	Significance ¹
PDM	Crop	4.24	1	0.039	*
	Moment	64.97	1	7.605×10^{-6}	***
	Irrigation	3.36	1	0.067	.
	N treatment	0.47	2	0.790	
PNC	Crop	14.64	1	0.00013	***
	Moment	9.68	1	0.0018	**
	Irrigation	0.00	1	0.964	
	N treatment	4.53	2	0.104	
NUp	Crop	0.40	1	0.525	.
	Irrigation	5.60	1	0.018	*
	N treatment	5.11	2	0.078	.
%N _c	Crop	3.58	1	0.058	.
	Moment	22.87	1	1.736×10^{-6}	***
	Irrigation	3.10	1	0.078	.
	N treatment	3.21	2	0.201	
NNI	Crop	1.09	1	0.298	
	Moment	17.19	1	3.381×10^{-5}	***
	Irrigation	3.83	1	0.050	.
	N treatment	3.47	2	0.177	

¹ Signif. codes: 0 “***”; 0.001 “**”; 0.01 “*”; 0.05 “.”; 0.1 “.”; 1. Blank cells in the “Significance” column indicate that the p-value did not reach the significance level.

Table 7. Dunn’s test results for the groups that presented significance in the Kruskal–Wallis test.

Parameter	Approach	z-Value	Adjusted p-Value	Significance ¹
NUp	N0 vs. N1	−2.2544	0.0363	*
	N0 vs. N2	−1.2223	0.3324	
	N1 vs. N2	0.9766	0.4931	

¹ Signif. Codes: 0.01 “*”; 0.05 “.”; 1. Blank cells in the “Significance” column indicate that the p-value did not reach the significance level. RG—annual ryegrass; Int—intercropping ryegrass-based; NNI—nitrogen nutrition index.

In these boxplots, the annual ryegrass crop has lower medians for PDM, PNC, and %N_c than the intercropped crop. Both PDM and PNC had higher medians in t2 compared to t1. These PNC values indicate that the crop was under stress and therefore lacking in nutrient N at t1. As can be seen in the %N_c boxplot, at t1 the PNC should be higher at earlier stages of the crop and decrease as the cycle progresses. The results also indicate that %N_c was higher in rainfed compared to irrigated conditions.

The boxplots in Figure 5 show that N absorption was higher than on rainfed land, with the medians presenting a difference of approximately 10 kg N ha^{−1}. The median value for irrigated NNI was also higher than for non-irrigated NNI. In terms of N treatments, N1 had the highest median NUp values, followed by N2. This indicates that the N absorbed by the plants was higher in the plots that were fertilized with 120 kg fertilizer ha^{−1}. Finally, the boxplot presenting the median NNI per moment indicates that the median NNI at the first sampling moment was just above 0.6 and at the second moment it was very close to 1,

corroborating the statement that the crops were in N deficit at t1, and were well nourished at t2.

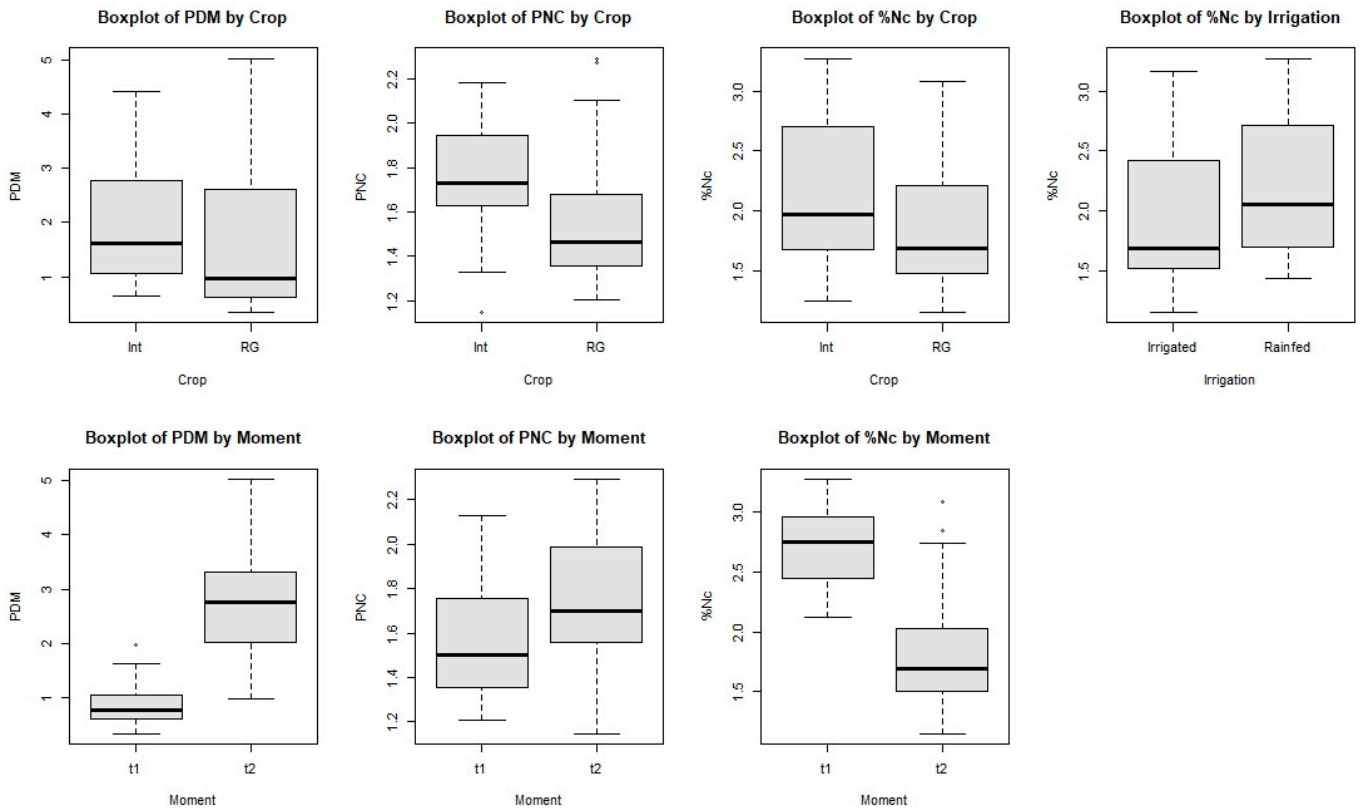


Figure 4. Boxplots of Crop and Moment groups for the PDM, PNC, and %N_c variables, and boxplot of Irrigation groups for %N_c.

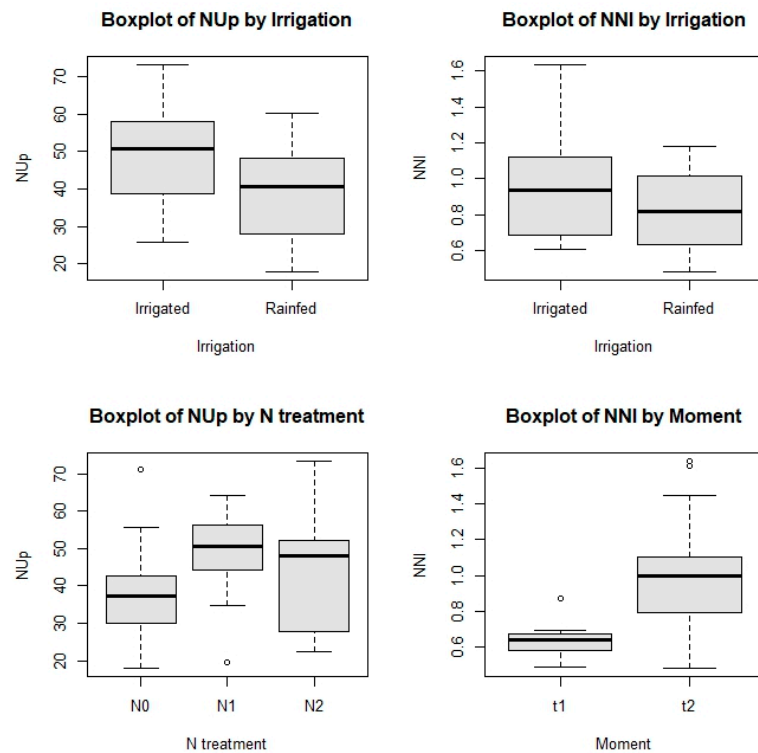


Figure 5. Boxplots of Irrigation groups for the variables NUp and NNI, boxplot of N treatment groups for NUp, and Moment groups for NNI.

3.2.2. Variables with Normal Distribution

The results of the ANOVA test indicate that, for the NUpE and NUE variables, there are no significant differences in the means between the groups based on the Crop, Irrigation, and N treatment factors.

For the NUE and NUpE variables (p -values < 0.05 in the Shapiro–Wilk test), there is not enough evidence to reject the null hypothesis of normality. ANOVA, which assumes that the model's residuals are normally distributed, was considered adequate and was applied to these two dependent variables (Table 8).

Table 8. Results of the ANOVA test for dependent variables that follow a normal distribution.

Dependent Variable	Factor	Df Group	Df Residual	Sum of Squares	Mean Squares	F-Value	p -Value	Significance ¹
NUpE	Crop	1	30	219.39	219.4	0.076	0.784	
	Irrigation	1	30	6641.09	6641.0	2.490	0.125	
	N treatment	1	30	1525.92	1526.0	0.538	0.469	
NUE	Crop	1	30	301.01	301.0	0.406	0.529	
	Irrigation	1	30	542.19	542.2	0.739	0.397	
	N treatment	1	30	1315.78	1315.8	1.859	0.183	

¹ Significance codes: 1. Blank cells in the “Significance” column indicate that the p -value did not reach the significance level.

Figure 6 presents the boxplots illustrating the results of the ANOVA test.

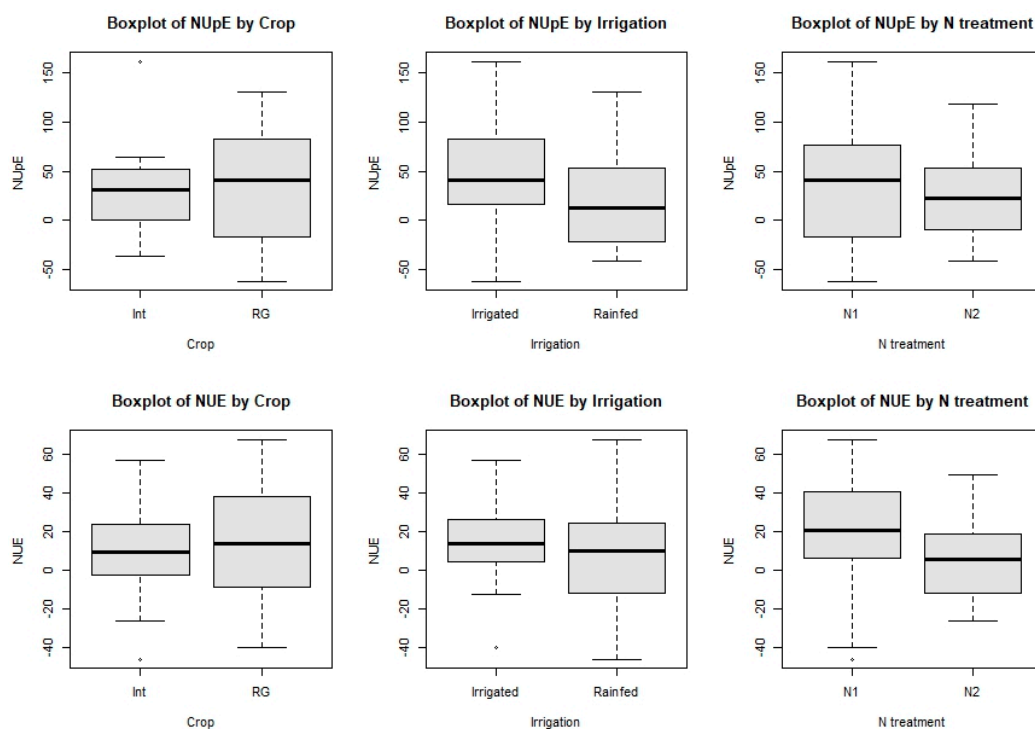
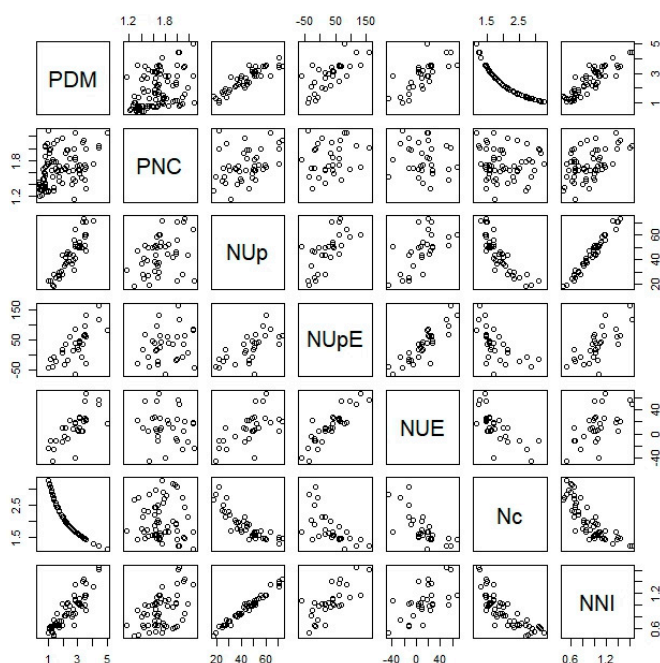


Figure 6. Boxplots of groups for the variables NUpE and NUE.

3.3. Relationships between NNI and N Use Parameters

To understand the relationship between the study's dependent variables, a correlation matrix was created. The results of the correlation matrix are presented in Figure 7.



	PDM	PNC	NU _p	NU _p E	NUE	%N _c	NNI
PDM	1.00						
PNC	0.39	1.00					
NU _p	0.89	0.35	1.00				
NU _p E	0.71	0.19	0.67	1.00			
NUE	0.73	-0.14	0.60	0.88	1.00		
%N _c	-0.95	-0.05	-0.85	-0.61	-0.70	1.00	
NNI	0.88	0.50	0.99	0.73	0.60	-0.83	1.00

(a)

(b)

Figure 7. Bivariate analysis of the dependent variables under study: (a) b-plots; (b) correlation matrix.

PDM presents a strong positive correlation (0.89) with NU_p. The results present a very strong inverse relationship (−0.95) between PDM and %N_c and a strong relationship (0.88) with NNI. NU_p has a strong inverse relationship (−0.85) with %N_c and an almost perfect relationship (0.99) with NNI. NU_pE has a strong positive correlation (0.88) with NUE. Finally, %N_c has a strong inverse relationship (−0.83) with NNI.

4. Discussion

4.1. CNDC

The use of the Bayesian statistical model was pivotal in establishing the CNDC in this study. The parameters A1 and A2, ranging between 3.18 and 3.87 and 0.53 and 0.72, respectively, are consistent with existing research, indicating the reliability of the determined CNDC ($\%N_c = 3.63 \times PDM^{-0.71}$). Compared to the curves of the references consulted and shown in Table 2, A1 is very close to the lowest A1 values, while A2 is close to the highest A2 values. A lower A1 value was recorded for oats (3.2) [28] and irrigated annual ryegrass (3.5) [28], and a very close value was recorded for timothy grass (3.7) [26]. This means that these were the G × M × E combinations with a critical N concentration value for sites with 1 t ha^{−1} most similar to the conditions in this trial. All the other crops considered as prior information registered a higher A1. In terms of A2, the value recorded was one of the highest, comparable only to the value achieved in perennial ryegrass by Gislum and Boelt [27]. All the other crops have lower A2 values and therefore a tendency for the N content in plant tissues to decrease more sharply. The decrease in %N_c is very slight. This insight allows the N fertilization manager to know that under optimum conditions the PNC should not drop sharply, and to make informed decisions to apply the nutrient in a way that meets the needs of the crop and the product it is intended to produce more sustainably.

The estimated curve allows us to know what the critical N concentration is at each moment of the crop cycle, and to be able to classify the real state of the crop. Two key aspects to bear in mind when applying this technique are the exclusion of data pairs with

a PDM lower than 1 t ha^{-1} , and the use of prior information. In this work, the removal of this data meant a considerable reduction in the number of samples available. Still, as identified by Makowski et al. [9], it is a necessary step to ensure that the curve fits practical agricultural scenarios. The value of $\%N_c$ changes with each $G \times M \times E$, meaning that there is an effortless way of using a short database, with free software, that can be used dynamically, thereby making it possible to calculate the most appropriate curve for each situation. This, as well as also knowing its level of uncertainty, was a great asset in this work, as already mentioned by others authors [9,10,31].

4.2. PDM and PNC

The initial statistical analysis revealed that outliers needed exclusion and most parameters did not follow a normal distribution, highlighting the variability in field experiments and the necessity for precision agriculture methods. This emphasizes the importance of data analysis, cleaning, and interpretation [9,10,31].

Comparing the two basic variables (PDM and PNC), PDM generally had a more asymmetrical distribution and dispersed values than PNC. Figure 4 confirms that PDM has the most values between 0.3 and 3.5 t ha^{-1} . Similar ranges have been reported as annual ryegrass (0.2 to 3.9 t ha^{-1} [6] and 0.1 to 3.9 t ha^{-1} [12]). The PNC distribution has most values between 1.2 and 2.3% . Sandaña et al. [6] observed a range of PNC in hybrid annual ryegrass of 1.6 to 5.1% and explained the variations of these traits by the N fertilization rates applied (0 , 50 , 100 , 200 , 350 , 525 , and 700 kg N ha^{-1}) and the harvest times. The maximum value was also slightly higher in the results achieved by Gislum et al. [27] in perennial ryegrass (0.8 to 4.6%). However, it should be borne in mind that Sandaña et al. [6] carried out the tests on clay soils with organic matter contents between 10.3 and 14.1% , and Gislum et al. [27] on fine sandy loam soils, and the N doses varied between 0 and 200 kg N ha^{-1} but were made in split applications.

The results indicate that the response of PDM and PNC varies with crops and sampling times. Annual ryegrass produced less PDM, averaging 1 t ha^{-1} , compared to the intercropped crop, which averaged 1.7 t ha^{-1} . However, the PDM values for annual ryegrass vary more and can reach the highest values. Additionally, annual ryegrass recorded lower PNC results than the intercropped crop, with values of 1.45% and 1.75% , respectively. These results corroborate the findings of Louarn et al. [23], indicating that intercropped crops require specific treatments tailored to their species. They help establish a benchmark for PDM and PNC results in these crops. The fact that PDM and PNC do not respond to the irrigation strategy and N treatments suggests that other factors, possibly soil properties or initial N status, play a more crucial role. More studies are needed to understand how different species compositions influence PDM and PNC production and the importance of N management strategies.

4.3. N Use Parameters and NNI

The results show that NUp (mean: 44.0 and range: 18 – 73 kg ha^{-1}) responds to the Irrigation factor. While the plants that were irrigated had an average NUp of 50 kg ha^{-1} , the rainfed plants had an average of 40 kg ha^{-1} . The results also show a different plant response between the N0 and N1 N treatments. Sandaña et al. [12] recorded NUp ranges between 4.9 and 134.0 kg ha^{-1} and between 3.7 and 101.0 kg ha^{-1} . In addition to achieving higher NUp values, they indicate that NUp responds mainly to N treatments, but also to harvest time.

NUPE (mean: 32.9 and range: -62 – 162%) and NUE (mean: 11.8 and range: -46 – $68 \text{ kg kg N ha}^{-1}$) did not respond to the studied crop groups Irrigation or N treatment. This suggests that these factors do not affect NUE and NUPE within the range studied, highlighting the potential for other agronomic practices to improve these efficiencies. Even so, the results show that in these $G \times M \times E$ scenarios, plants absorb an average of 32.9% of the total N from the fertilizer. This absorption efficiency means that, on average, for every kg of N applied, the crop produces 11.8 kg more PDM. Sandaña et al. [12] indicate

that under their experimental conditions, annual ryegrass produces an average of 36.6 kg of PDM per kg of N absorbed. This result is close to the 40.5 kg of PDM achieved in this study. Marino et al. [11] achieved similar results when applying 50 kg N ha⁻¹ (44.2 and 52.2 kg PDM kg⁻¹), and lower output when applying a higher dose of 250 kg N ha⁻¹ (17.61 and 15.02 PDM kg⁻¹).

The %N_c value decreases as the crop cycle progresses, from 3.2% at t1 to 1.2% at t2. %N_c shows minimum values (1.2%) equal to those of the PNC achieved but shows a maximum value (3.2%) higher than the maximum value shown in the PNC (2.3%). The average %N_c (2.0%) is also higher than the average PNC (1.66%). These results indicate that critical N concentrations were not reached. These results are reflected in the NNI results presented below. Other authors [12] have recorded higher %N_c values in the early stages of crop cycles, reaching 4.6%. The %N_c responds very well to the Moment and the average decreases from 2.8% at t1 to 1.7% at t2. This result means that these would be the optimum PNC values for the crops to be in the optimum nutritional state and to ensure that the crops express their productive potential. However, in t1, the PNC value was well below the %N_c value, reaching only 1.5%. In t2, the average PNC value already reached the %N_c value. This difference between PNC and %N_c translates into an average NNI of 0.65 in t1 and 1.00 in t2. The NNI responds not only to Moment but also to Irrigation. The NNI values achieved in this study (mean: 0.91 and range: 0.48–1.63) indicate that the plants were in deficit of the macronutrient in the Moment t1. In future studies, changes will be made to guarantee the good nutrition of the plants in the crucial first stages of development. Similar ranges of values were achieved by other authors in annual ryegrass (0.4–1.6) [11].

4.4. Correlations

The correlation matrix reveals strong relationships between PDM, NUp, %N_c, and NNI, emphasizing the interconnectedness of biomass production and N dynamics. The PDM presents a strong positive correlation (0.89) with NUp, indicating that biomass production is closely linked to the amount of N absorbed by the crop. The solid inverse relationship (−0.95) between PDM and %N_c indicates that an increase in biomass production is linked to a decrease in the critical N concentration in the crop. The base knowledge identified by other authors [4–7,22,25] was verified, and PDM increases exponentially as the crop cycle progresses and the crop's optimal N content decreases exponentially. The PDM also has a strong relationship (0.88) with NNI, indicating that plants with a higher NNI produce more biomass [32].

The almost perfect correlation between NUp and NNI highlights that higher NUp value enhances NNI, reinforcing the importance of efficient N management to benefit the N absorption by the plants. Similar insights were achieved in intercropping fodder [33]. The strong correlation between NUpE and NUE indicates that improving NUpE directly enhances overall NUE, which is crucial for sustainable agriculture, following other authors [12,32,34].

4.5. Implications for Practical Ryegrass-Based Fodder Crop Production

Implementing the CNDC as a diagnostic tool in fodder crop production is highly appealing. The CNDC should be used to determine if additional N application is necessary to increase PNC above the CNDC at the current biomass level. An increase in PNC through additional N application should ensure that PNC is not a limiting factor for achieving a high PDM yield. Focus should therefore be on using the CNDC in the period of growth (initial stages of crop development) where an application of N has a positive effect on the relative growth rate and the amount of N accumulated in the plant.

The analysis indicates that PDM and NUp are strongly interlinked with several other variables, highlighting the importance of proper N management to optimize crop production. In particular, NUpE and NNI in the plant have strong associations that should be considered in agricultural management practices. These relationships highlight the

complex interaction between biomass production and the N cycle in plants, providing valuable insights for improving the efficient use of this nutrient.

Additionally, this approach will help define reference values for N use parameters under optimal N nutritional status, such as $\%N_c$ to guarantee a perfect N nutritional status (NNI = 1) of ryegrass-based fodder crops at each key stage of crop development, and NUE (11.8 kg kg N ha⁻¹) and NUpE (32.9%) to know what to expect in terms of the use of N fertilizer by the plants. These reference values can be used to benchmark N management practices aimed at improving NUE in sustainable agricultural systems.

These insights highlight the high variability and wide range in the studied parameters, and underscore the complexity of N dynamics in cropping systems and the need for integrated management approaches. These findings are critical for devising precision N management practices that consider the specific needs of different crops and their growth stages, ultimately enhancing NUE and crop productivity.

5. Conclusions

This study established a CNDC using a Bayesian model, providing a tool for predicting N requirements and optimizing fertilizer application in annual ryegrass-based forage crops. The CNDC is defined as $\%N_c = 3.63 \times PDM^{-0.71}$. The results demonstrated that PDM and PNC varied significantly across different crop types and sampling moments, with strong positive correlations between PDM and NUp (0.89) and NNI (0.88), and an inverse correlation with $\%N_c$ (−0.95).

The study recommends the following practices:

- Precision N management: Implement the CNDC to predict N requirements accurately and optimize fertilizer application.
- Irrigation practices: Prioritize effective irrigation as it significantly enhances NUp and NNI, thus improving NUE. Crops under irrigation conditions showed higher NUp and NNI, indicating that water management is crucial for improving NUE.
- Differentiated N doses: Adjust N doses based on crop type, growth stages, and specific field conditions to maximize NUE.

Future research should focus on the long-term impacts of these practices on soil health and explore the integration of N management with other nutrient strategies, as well as technological innovations such as remote sensing and machine learning for real-time monitoring of crop nutritional status.

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