



Reducing the interval between termination of a legume cover crop (Persian clover) and rice sowing increases soil mineral nitrogen and plant development

Cristiano Weinert · Ezequiel Helbig Pasa · Verônica Lemos Vargas · Sidnei Deuner · Júlia Peralta Ferreira · Rogério Oliveira de Sousa · Filipe Selau Carlos

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Abstract The cultivation of Persian clover (*Trifolium resupinatum* L.) is an alternative to minimize the use of nitrogen (N) fertilizers in irrigated rice (*Oryza sativa* L.) and achieve economic and environmental sustainability in floodplain areas. The present study aims to evaluate the effect of chemical termination times of Persian clover on the dry matter (DM) yield of Persian clover and irrigated rice and nutrient availability in the soil solution. The experiment was conducted in a greenhouse in 2019/2020 and 2020/2021, in a completely randomized experimental design. Factor 1 consisted of Persian clover termination intervals (45, 30, 15, and 0 days before rice sowing), factor 2 consisted of N rates (0 and 150 kg N ha⁻¹) supplemented with urea (46% N), and two fallow treatments without Persian clover were included for comparison. Dry matter production,

nutrient concentrations in clover and rice, soil solution nutrients, dissolved organic carbon (DOC), and N-ammonium and N-nitrate were measured. Clover DM increased by 1.7% for each day that termination was delayed toward rice sowing, with the highest DM observed when clover was terminated at sowing (C0) and the lowest when terminated 45 days before sowing (C45). DOC, N-ammonium, and N-nitrate in the soil solution were 83.2, 79.8, and 57.4% higher, respectively, in plots previously cultivated with Persian clover than in the fallow treatments. These results indicate that terminating clover at rice sowing enhances dry matter production and nutrient availability, supporting improved performance of the subsequent irrigated rice crop.

Keywords *Trifolium resupinatum* L. · Soil solution · Efficiency · Cover crops

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C. Weinert (✉) · E. H. Pasa · V. L. Vargas
Postgraduate Program in Soil and Water Management and Conservation, Federal University of Pelotas, Capão do Leão, Brazil
e-mail: cristianoweinert@gmail.com

S. Deuner · J. P. Ferreira
Federal University of Pelotas, Capão do Leão, Brazil

R. O. de Sousa · F. S. Carlos
Professor Soil Science Department, Federal University of Pelotas, Capão do Leão, Brazil

Introduction

The significant increase in the yield potential of irrigated rice has been associated with genetic improvement and management practices, particularly the high requirement for nitrogen (N) fertilization (Carmona et al. 2016). In South America, soils cultivated with irrigated rice often have low N concentration, and the application of 150–180 kg N ha⁻¹, is considered optimal to achieve high yields (Carlos et al. 2022). However, while these rates support high productivity, they

can also lead to excessive N losses, contaminating rivers and lakes through eutrophication, and increasing production costs (Barr et al. 2020; Beaulieu et al. 2019).

In flooded rice systems, N dynamics are strongly influenced by soil redox conditions, residue decomposition, and the balance between mineralization and immobilization processes (Sousa et al. 2009; Ponnampersuma 1977). Therefore, strategies that better synchronize N release from organic residues and crop demand are essential to improve N use efficiency and reduce environmental impacts (Crews and Peoples 2005). The synchronization of residue decomposition with crop N uptake depends largely on residue biochemical composition, especially the carbon-to-nitrogen (C:N) ratio and the presence of labile organic compounds (Peoples et al. 2009; Tripolskaja et al. 2023). Legumes typically present low C:N ratios and high concentrations of amino acids and proteins, which promote rapid mineralization and increase mineral N availability shortly after termination. However, if residue decomposition occurs too early or too late relative to crop establishment, nutrient release may become asynchronous with crop demand, reducing nutrient recovery efficiency and increasing the risk of losses (Crews and Peoples 2005).

In this context, cover crops, particularly legumes, have been recognized as an effective strategy to supply N biologically, improve soil structure, reduce erosion, and enhance nutrient cycling in irrigated rice systems (Peoples et al. 2017; Veloso et al. 2018). However, the establishment of legumes during the off-season is challenging in lowland rice fields due to waterlogging and poor drainage, which restricts the choice of suitable species and limits their adoption in conventional cropping systems (Sousa et al. 2021; Menezes et al. 2001).

Among potential cover crops, Persian clover (*Trifolium resupinatum* L.) has gained attention due to its tolerance to waterlogged conditions, high dry matter production (4–6 Mg ha⁻¹), and early development (Weinert et al. 2023). In upland conditions, the benefits of winter legumes in crop rotations are well documented, contributing to increases in soil organic carbon (Veloso et al. 2018) and soil mineral N concentration (Peoples et al. 2017). These effects are related to the characteristics of legumes, such as high tissue N content and low carbon-to-nitrogen (C:N) ratio, typically ranging from 10:1 to 20:1, which

promote residue mineralization and N availability for subsequent crops (Peoples et al. 2017; Tripolskaja et al. 2023).

Beyond N, winter legumes can also influence the cycling and availability of other macronutrients, such as phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg). Residue decomposition contributes to the release of these nutrients into the soil solution and may alter their temporal availability during early crop development (Xavier et al. 2017; Veloso et al. 2018). In flooded systems, changes in redox potential can further affect nutrient solubility and mobility, influencing plant uptake patterns (Sousa et al. 2009). Therefore, evaluating only N dynamics may provide an incomplete understanding of the effects of legume termination timing on subsequent rice nutrition.

Additionally, residue decomposition can modify soil dissolved organic carbon (DOC) dynamics, which play a central role in microbial activity, nutrient mineralization, and redox reactions in submerged soils (Jiang et al. 2021; Sousa et al. 2009). Increases in labile organic carbon from legume residues may stimulate microbial growth and enzymatic activity, accelerating the mineralization of organic N and enhancing ammonium availability in flooded systems. Moreover, greater microbial respiration can intensify oxygen depletion and shift redox conditions, potentially influencing the solubility and mobility of nutrients such as P, Fe, and Mn (Ponnampersuma 1977; Sousa et al. 2009). Thus, assessing DOC and nutrient concentrations in the soil solution is essential to elucidate the mechanisms underlying plant responses.

Despite these benefits, the use of winter legumes in lowland rice is limited, and ryegrass (*Lolium multiflorum* L.) remains the most widely used winter cover crop in these conditions due to its greater tolerance to temporary waterlogging, ease of establishment under poorly drained soils, and well-established management practices in southern Brazil (Sousa et al. 2021). Ryegrass has a high C:N ratio at the end of the cycle (typically ranging from 30:1 to 60:1), which can restrict N availability and temporarily limit the cycling of other nutrient for the following rice crop. Under minimum cultivation, ryegrass is typically chemically managed 30–40 days prior to rice sowing, particularly when dry matter production exceeds 5 Mg ha⁻¹ (SOSBAI 2022). Similarly, Persian clover is managed chemically in some areas to prevent potential negative effects of organic acid

accumulation from residue decomposition during flooding (Sousa 2001). However, as a legume species, Persian clover typically presents a lower C:N ratio, faster residue mineralization, and greater N release compared with grasses such as ryegrass, which can enhance N availability for the subsequent crop (Peoples et al. 2009; Crews and Peoples 2005).

Therefore, we hypothesize that terminating Persian clover closer to rice sowing increases its biomass and biological N fixation, enhancing nutrient inputs to the system and improving synchronization between residue mineralization and rice nutrient demand. This effect may influence not only N availability, but also the dynamics of P, K, Ca, Mg, and DOC in the soil solution, ultimately affecting rice physiology, growth, and aboveground biomass. Therefore, the objective of the present study was to evaluate the effect of timing of Persian clover termination on its DM production, nutrient, and DOC dynamic in the soil solution, as well as nutrient accumulation, chlorophyll indices and aboveground biomass of the subsequent irrigated rice.

Material and methods

Site description, experiment design and management practices

The experiment was carried out in a greenhouse at the Federal University of Pelotas (UFPEL), located in the municipality of Capão do Leão-RS, coordinates 31°48'04.07" S and 52°24'41.23" W, for two crop seasons, in 2019/2020 and 2020/2021. The experimental design was completely randomized (CRD) with 4

repetitions, in factorial 4 (factor 1) × 2 (factor 2) + 2 (fallow), consisting of 40 experimental units. Factor 1 consisted of 4 intervals of chemical management of Persian clover (45, 30, 15 and 0 days before rice sowing), while factor 2 consisted of two rates of N fertilizer: 0 and 150 kg N ha⁻¹ as urea (46% of N). Two fallow treatments were used, with rates of 0 and 150 kg N ha⁻¹, in order to evaluate the effect of nutrient availability without Persian clover cultivation on the development of irrigated rice. The description of the treatments and coding are in Table 1. The experimental units consisted of 12 L black plastic pots with 11 kg of dry soil. The soil used was a Planosol (WRB; IUSS Working Group 2015) collected in the municipality Capão do Leão, with the following chemical characteristics (Tedesco et al. 1995): soil organic matter (SOM): 19.3 g kg⁻¹, clay: 20%, pH_{H2O} (1:1): 4.8, ECEC: 4.8 cmol_c dm⁻³, CEC_{pH 7}: 9.5 cmol_c dm⁻³, Ca: 2.6 cmol_c dm⁻³, Mg: 1.2 cmol_c dm⁻³, Al: 0.8 cmol_c dm⁻³, H + Al: 5.5 cmol_c dm⁻³, V: 42%, P: 16.2 mg dm⁻³, K: 47 mg dm⁻³ (Ca, Mg and Al were extracted with 1 mol L⁻¹ KCl; H + Al was determined by the SMP buffer method; P and K determined by Mehlich⁻¹).

The pH correction was carried out according to the technical recommendations of the Soil Chemistry and Fertility Commission—RS/SC (2016), raising the pH to 6.0 using limestone with 85% of effective calcium carbonate equivalent (ECCE). As a supply of P and K, rates of 78.5 kg P ha⁻¹ (180 kg P₂O₅ ha⁻¹) using triple superphosphate and 124.5 kg K ha⁻¹ (150 kg K₂O ha⁻¹) using potassium chloride, respectively, were used, which were incorporated into the soil together with the limestone. After incorporation, soil solution extractors were installed in the pots, according

Table 1 Description of the treatments used in the experiment

Treatment code	Description
F_N0	Fallow (no cover crop) + 0 kg N ha ⁻¹
F_N150	Fallow (no cover crop) + 150 kg N ha ⁻¹
C0_N0	Persian clover terminated at sowing (0 DBRS) + 0 kg N ha ⁻¹
C0_N150	Persian clover terminated at sowing (0 DBRS) + 150 kg N ha ⁻¹
C15_N0	Persian clover terminated 15 DBRS + 0 kg N ha ⁻¹
C15_N150	Persian clover terminated 15 DBRS + 150 kg N ha ⁻¹
C30_N0	Persian clover terminated 30 DBRS + 0 kg N ha ⁻¹
C30_N150	Persian clover terminated 30 DBRS + 150 kg N ha ⁻¹
C45_N0	Persian clover terminated 45 DBRS + 0 kg N ha ⁻¹
C45_N150	Persian clover terminated 45 DBRS + 150 kg N ha ⁻¹

DBRS: days before rice sowing

to the methodology of Sousa et al. (2002), at depths of 5 and 10 cm. The Persian clover cultivar *Lightning* was used at a density of 40 seeds per pot, which were thinned immediately after emergence to 10 plants per pot (density of 4.5 kg seeds ha⁻¹). The IRGA 424 RI rice cultivar was used, with 8 seeds per pot sown and also thinned immediately after emergence to 2 rice plants per pot (density of 40 kg seeds ha⁻¹). Nitrogen fertilization was divided into two applications, 67% in stage V₃ (beginning of flooding) and 33% in stage V₉ (nine established leaves) (SOSBAI 2022). In the second year, the same pots cultivated in the first year were used, simulating the Persian clover-rice succession, aiming to evaluate the effect of the accumulation of crop residues on the dynamics of nutrients in the soil solution.

Dry matter biomass

The quantification of DM of Persian clover was performed by collecting one representative plant per pot in each chemical management interval, and the remaining plants were kept to not interfere with the chemical management performed in each treatment. After collection, the representative plant was dried in an oven at 65°C for 3 days and subsequently weighed. The value obtained consisted of the weight of dry matter of a plant, which was extrapolated to the other plants and converted into dry matter production per pot.

Shoot nutrient concentration

The nutrient concentrations of the dry matter of Persian clover and rice plants were determined by collecting plant tissue samples as described in Sect. “**Dry matter biomass**”, grinding each sample, and weighing 0.2 g of the ground tissue through a 2 mm sieve from each experimental unit. The samples were then subjected to acid digestion using 2 mL of PA-grade sulfuric acid (H₂SO₄), 1 mL of PA-grade hydrogen peroxide (H₂O₂), and 0.7 g of a digestion mixture (200 g of anhydrous sodium sulfate [Na₂SO₄] and 20 g of copper (II) sulfate pentahydrate [CuSO₄·5H₂O]). The digestion procedure consisted of heating at 255 °C for 1.5 h followed by 336 °C for 1 h. After digestion, the samples were brought to a final volume of 50 mL (Tedesco et al. 1995). After digestion, the total N concentration was determined by the *Kjeldahl* distillation

method, the K concentration by flame emission spectrophotometry, the P concentration by visible spectrophotometry, and the calcium (Ca) and magnesium (Mg) concentrations by atomic absorption (Tedesco et al. 1995).

Soil solution collection

The soil solution was collected using a 70 cm long polyethylene tube with an internal diameter of 0.6 cm, perforated with two rows of 1 mm holes, lined with nylon screen (400 mesh) to prevent the entry of fine soil particles. The tube was shaped into a spiral, with 2 cm between turns, and connected to a glass siphon to facilitate the removal of the solution without strong suction, avoiding clogging and attracting particles. At the end of the siphon, a 2 cm silicone tube closed with a glass stopper was attached (Sousa et al. 2002). The spirals were installed at 5 and 10 cm depth, before sowing Persian clover. Weekly collections were carried out for eight weeks after flooding by connecting the soil solution collection device to 60 mL plastic syringes, which were used to generate negative pressure and extract the soil solution by suction.

After collection, the samples were filtered through a 0.45 mm Millipore membrane filter, approximately 50 mL of the soil solution filtrate were acidified with 1 mL of 3 mol L⁻¹ HCl and stored in sealed vials until analysis. Dissolved organic carbon (DOC) concentration was determined by the Walkley–Black method, N-ammonium and N-nitrate concentrations were quantified and determined by the *Kjeldahl* distillation method, P by visible spectrophotometry, K by flame emission spectrophotometry, and Ca and Mg by atomic absorption spectrophotometry, according to the procedures described by Tedesco et al. (1995). The average values obtained from the two sampling depths were used to calculate nutrient concentrations.

Physiological indices

At the R0 (panicle initiation) phenological stage (Counce et al. 2000), immediately after the second N fertilization, the chlorophyll, flavonoid, anthocyanin and N balance indices were determined in the rice plants, where the N balance index is calculated as the ratio between the chlorophyll index and the anthocyanin index, providing an indirect indicator of the plant nitrogen status. The readings were performed in the

middle third of the plants (Yuan et al. 2016), with the aid of a Dualex FORCE-A chlorophyll meter, and the values obtained consisted of the average of one reading per leaf, two leaves per plant, and two plants per pot, totaling 4 readings in each pot and 16 readings per treatment.

Rice height, tillers and dry matter production

Tiller counts were performed before cutting to determine dry matter. The tillers of the two plants (one count per plant) were counted and the average was used for calculations. The height of the rice plants was determined using a tape measure, where two measurements of the rice plant (i.e., one measurement per rice plant) were taken for each pot. The average of the two measurements was used in the calculations. The dry matter of the rice plants was obtained by cutting both plants at the R_1 (beginning of flowering) stage and subjecting them to the same drying process as the clover. The value was expressed in dry matter production per pot.

Statistical analyses

The data were evaluated with the Shapiro–Wilk method to verify the normal distribution of residues. For the data on production and nutrient content of the DM of Persian clover, analysis of variance was performed following the assumptions of normality and, when significant ($p \leq 0.05$), the data were adjusted to the linear and quadratic regression model ($p \leq 0.05$), with the absence of the fallow treatment. Year was included as a fixed factor in the analysis, together with the termination time and N rate, to test their interactions. When significant interactions involving year were observed, the data were analyzed separately for each crop season. For soil solution variables, when significant interactions between termination time and N rate were observed within each season, a simple-effects analysis was performed, and termination times were compared within each N rate using Tukey's HSD test ($p \leq 0.05$). For the remaining variables, termination times and N rates were compared within each crop season using Tukey's HSD test ($p \leq 0.05$).

The soil solution data were presented as a function of the termination times and N rates over the flooding period due to significant interaction between

these factors. The remaining variables and statistical analyses were performed with the Tukey HSD test ($p \leq 0.05$), using the ExpDes package (Ferreira et al. 2013). Pearson correlation was performed using the R packages corrplot (Wei and Simko 2021) and Hmisc (Harrell and Harrell 2019). All analyses were performed with the aid of the R® program (R Core Team 2020).

Results

Persian clover dry matter biomass

In both crop seasons (2019/20 and 2020/21), DM of Persian clover increased with shorter termination intervals, showing a significant linear relationship between DM biomass and termination interval (Fig. 1). In the first season, DM increased by 1.6% per additional day of growth. In the second season, this increase was more pronounced, reaching 1.7 and 1.8% per day in the 150 and 0N treatments, respectively.

Nutrients in Persian clover dry matter biomass

The accumulation of N, K, P (Fig. 2), and Ca and Mg (Fig. S1) in Persian clover dry matter showed quadratic responses to the termination intervals in both crop seasons, with higher nutrient accumulation at later termination (0 and 15 DBRS), resulting primarily from higher biomass, while nutrient concentrations were maintained or higher at earlier termination (30 and 45 DBRS). Across nutrients, the clover terminated at sowing (0 DBRS) consistently had the highest accumulations, highlighting its role as a nutrient-rich cover crop.

In 2019/2020, the accumulation of N (Fig. 2a), K (Fig. 2c), and Ca (Fig. S1) increased progressively with decreasing termination intervals, while P accumulation (Fig. 2e) showed a distinct response, where the treatment without N fertilization presented higher values at 0 DBRS compared with the fertilized treatment. Similarly, Mg accumulation (Fig. S1) was lower with earlier termination (45 DBRS), and variations in accumulation were primarily driven by differences in biomass rather than changes in tissue Mg concentration (Table S1).

In 2020/2021, nutrient accumulation generally increased when Persian clover was terminated closer

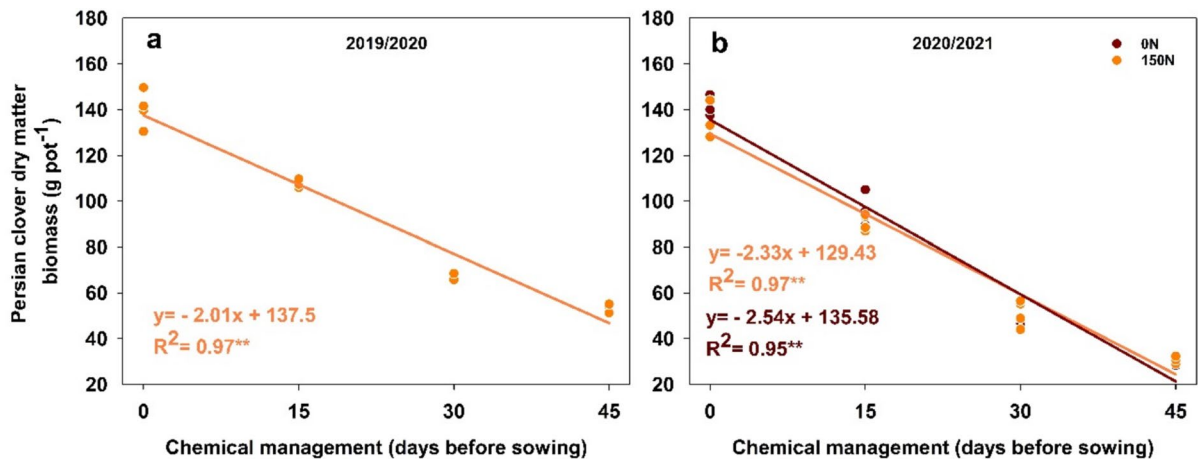


Fig. 1 Persian clover dry matter biomass, in the crop seasons 2019/2020 (a) and 2020/21 (b), under different times of chemical management before rice sowing

to rice sowing, although some differences compared with the previous year were evident. Nitrogen accumulation (Fig. 2b) was higher at 0 and 15 DBRS, and P (Fig. 2f) also showed higher accumulation compared with 2019/2020, indicating a seasonal effect on nutrient uptake and biomass production. In contrast, tissue nutrient concentrations (Table S1) did not show a consistent decreasing trend with delayed termination. Although earlier termination (30 and 45 DBRS) resulted in higher concentrations of some nutrients, greater nutrient accumulation at later termination (0 and 15 DBRS) was primarily driven by increased dry matter production rather than changes in tissue concentration. The effect of N fertilization on nutrient concentrations was nutrient- and year-dependent, being more evident for P in 2019/2020 and for N and Mg in 2020/2021.

Nutrient and DOC dynamics in soil solution

In 2019/2020, descriptive temporal patterns (i.e., not analyzed statistically) indicated that treatments C30_N150 and C15_N150 showed higher DOC concentrations in the soil solution at 1 day after flooding (Fig. 3a). From the 21st to the 36th day after flooding, DOC concentrations were visually higher in treatments C0_N0, C0_N150, C15_N0, and C15_N150 compared with the other treatments; thereafter, DOC levels stabilized at approximately 5 mg L⁻¹ (Fig. 3a). In 2020/2021, temporal trends showed that the highest DOC concentrations occurred in treatments

C0_N0 and C0_N150 from the 1st to the 22nd day after flooding, whereas after the 43rd day, treatments C15_N0 and C15_N150 exhibited higher DOC concentrations (Fig. 3b).

When DOC concentrations were averaged over the first 29 days after flooding and analyzed statistically (Table 2), C0_0 showed the highest mean DOC values among the non-fertilized treatment in both crop seasons. Under N fertilization (150 kg N ha⁻¹), the highest average DOC concentrations were observed in C15_N150 in 2019/2020 and in C0_N150 in 2020/2021.

In both growing seasons, descriptive temporal patterns showed that treatments C0_N0 and C0_N150 had the highest N-ammonium concentrations during the flooding period (Figs. 3c and 3d), especially when compared to treatments with early desiccation of the Persian clover. In the 2019/2020 season, N-nitrate concentrations were higher at 1 day after flooding in treatments C45_N0 and C45_N150 (Fig. 3e). In the 2020/2021 crop season, N-nitrate concentrations during the early flooding period (up to the 8th day after flooding) were higher across all treatments, regardless of the N rate compared with those observed in the 2019/2020.

When averaged over the first 29 days of flooding and analyzed statistically, N-ammonium concentrations were significantly higher in treatments C0_N0 and C0_N150 than in the other treatments in both crop seasons (Table 2). For N-nitrate, when averaged over the first 29 days after flooding,

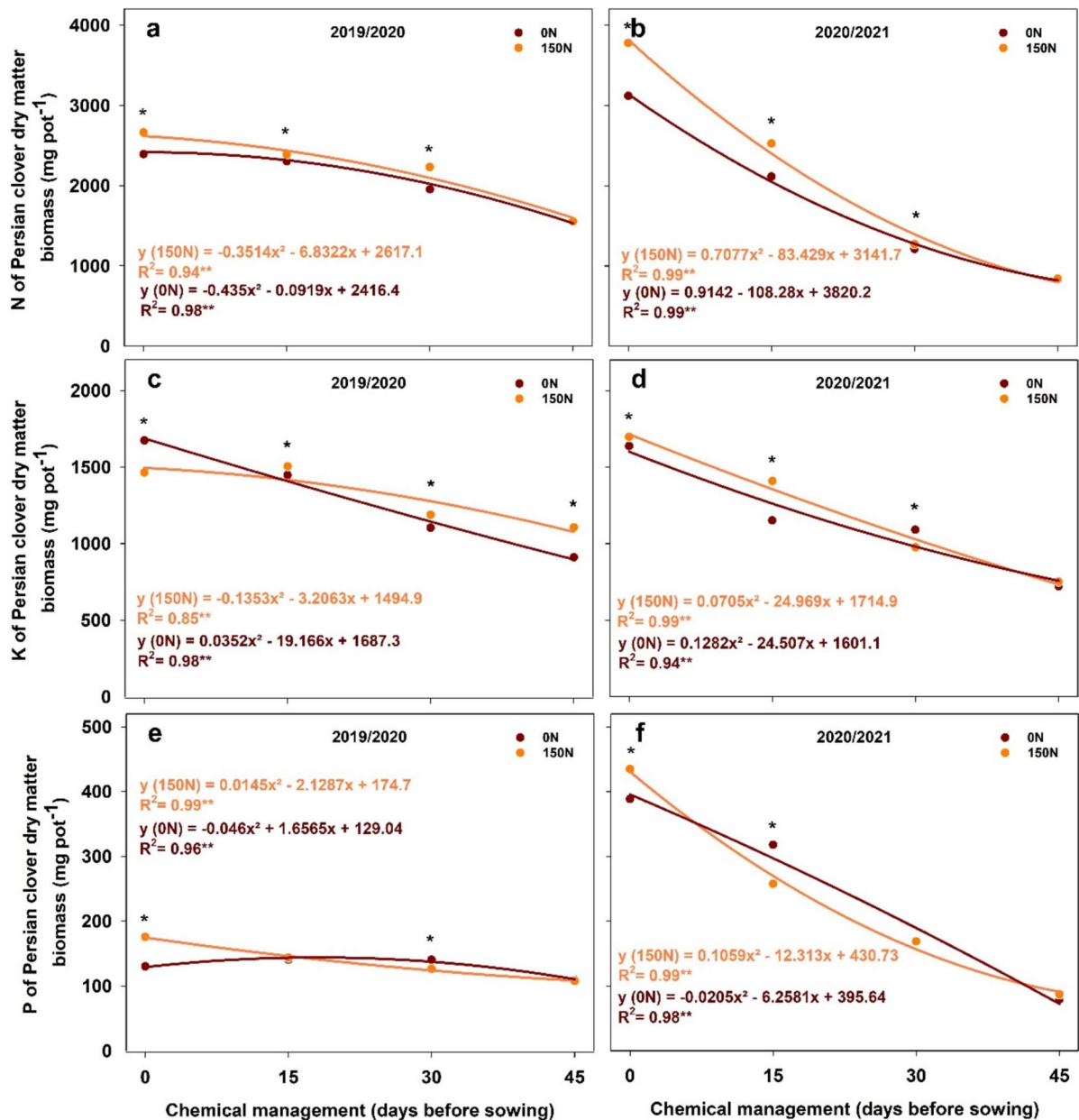


Fig. 2 Nitrogen in 2019/2020 (a) and 2020/21 (b), potassium (K) in 2019/2020 (c) and 2020/2021 (d), and phosphorus (P) in 2019/2020 (e) and 2020/2021 (f) of Persian clover dry matter biomass in Planosol in the 2019/2020 and 2020/2021 crop seasons, in a greenhouse under different times of chemical management; clover 0—termination of Persian clover at the

time of rice sowing; clover 15—termination of Persian clover 15 days before rice sowing; clover 30—termination of Persian clover 30 days before rice sowing and clover 45—termination of Persian clover 45 days before rice sowing. *asterisks indicate significant difference by the Tukey test ($p < 0.05$) within each clover termination period in relation to the use of N or not

higher concentrations were observed in C45_N0 in 2019/2020 and C45_N150 in 2020/2021, indicating a significant effect of longer clover termination intervals (Table 2).

In the 2019/2020 season, P concentration in the soil solution remained at similar levels throughout the season (Fig. 4a). In the second season, descriptive temporal patterns indicated that treatments C0_N0

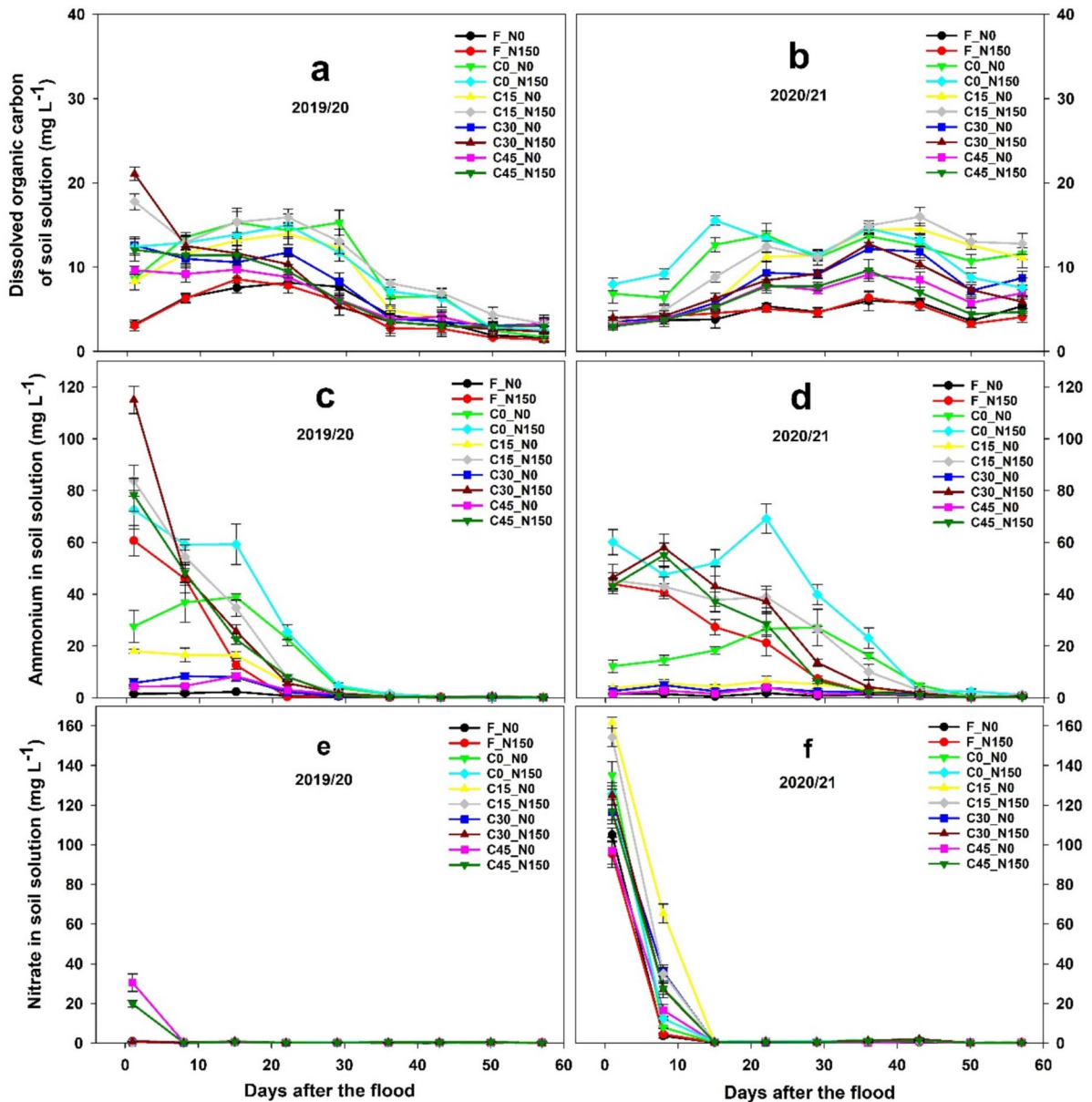


Fig. 3 Dissolved organic carbon (DOC) concentration in the soil solution in the 2019/20 (a) and 2020/21 (b) growing seasons, ammonium in the soil solution in the 2019/20 (c) and 2020/21 (d) growing seasons, and nitrate in the soil solution in

the 2019/20 (e) and 2020/21 (f) growing seasons, as a function of different Persian clover termination periods with and without nitrogen application, during the flooding period

and C0_N150 had higher P peaks at 8 and 15 days after flooding (Fig. 4b). When averaged over the first 29 days after flooding, P concentrations were numerically higher in 2020/21 than in 2019/20 (Table 2).

Soil solution K concentrations showed similar descriptive temporal patterns in both seasons, with

higher values in the first days after flooding and a progressive decrease thereafter (Fig. 4c and d). In 2019/2020, higher K concentrations immediately after flooding were observed in treatments C30_N0, C30_N150, C45_N0, and C45_N150 (Fig. 4c). In the 2020/2021 season, treatments Fallow_N150 and

Table 2 Average concentration of dissolved organic carbon (DOC), ammonium, nitrate, phosphorus, potassium, calcium and magnesium in the soil solution during the first 29 days after flooding, at rates of 0 and 150 kg N ha⁻¹ in the 2019/2020 and 2020/2021 crop seasons, under different times of chemical management of Persian clover: Fallow—without Persian clover

Treatments		DOC		Ammonium		Nitrate		Phosphorus		Potassium		Calcium		Magnesium	
Crop season		19/20	20/21	19/20	20/21	19/20	20/21	19/20	20/21	19/20	20/21	19/20	20/21	19/20	20/21
		mg L ⁻¹													
0N	Fallow	6.6 e	4.1 d	1.4 d	1.3 c	0.3 b	21.9 c	1 b	3.1 c	7.7 d	16.1 a	147 d	145 c	92 c	103 b
	C0_N0	13.4 a	10.2 a	26 a	19.8 a	0.5 b	28.9 c	0.9 c	5.6 a	11.2 d	13.5 a	172 c	153 b	108 a	105 ab
	C15_N0	11.9 b	7.1 b	11.7 b	5.1 b	0.3 b	45.7 a	1.1 a	3.8 c	17.5 c	6.6 c	169 c	162 a	105 a	110 a
	C30_N0	10.8 c	6.3 b	5 c	3.3 bc	0.2 b	30.6 b	1.1 a	3.6 c	21.7 b	10.5 b	207 a	158 ab	107 a	107 ab
	C45_N0	8.7 d	5.5 d	4.7 cd	2.3 bc	6.4 a	23 c	1.1 a	4.6 b	29.4 a	13.5 a	192 b	156 ab	98 b	105 ab
150N	Fallow	6.3 d	4.3 e	24.1 d	28.1 d	0.4 b	20.1 c	0.9 c	3.1 bc	8.8 c	18.7 a	162 d	164 a	96 b	111 ab
	C0_N150	13.2 b	11.5 a	44.2 a	53.7 a	0.5 b	28.1 b	1 b	4.8 a	11.3 bc	18.1 a	166 d	149 b	103 b	104 c
	C15_N150	15.1 a	8.2 b	36.6 b	38.3 bc	0.4 b	38.2 a	1.1 a	3.3 b	15.2 b	9 c	223 a	163 a	112 a	110 ab
	C30_N150	12.2 b	6.4 c	39.1 b	39.6 b	0.4 b	30.7 b	1.1 a	2.6 c	22.2 a	18.3 a	194 b	169 a	99 b	114 a
	C45_N150	10.1 c	5.5 d	31.7 c	34.1 c	4.2 a	29.1 b	1 b	3.4 b	25.3 a	13.5 b	181 c	164 a	99 b	108 bc

Means followed by the same letter within each crop season and N rate do not differ from each other according to Tukey's test ($p < 0.05$). Ns: no significant difference

C30_N150 had the highest K concentrations during the first week after flooding (Fig. 4d). When averaged over the first 29 days after flooding, significant differences in K concentrations were observed among treatments within each crop season and N rate. In 2019/2020 at 150 kg N ha⁻¹, C30_N150 and C45_N150 showed the highest K concentrations, whereas in 2020/2021 the highest values were observed in Fallow_N150, C0_N150 and C30_N150 (Table 2). At 0 kg N ha⁻¹, C45_N0 presented the highest mean K concentration in 2019/2020 and remained among the statistically highest treatments in 2020/2021 (Table 2).

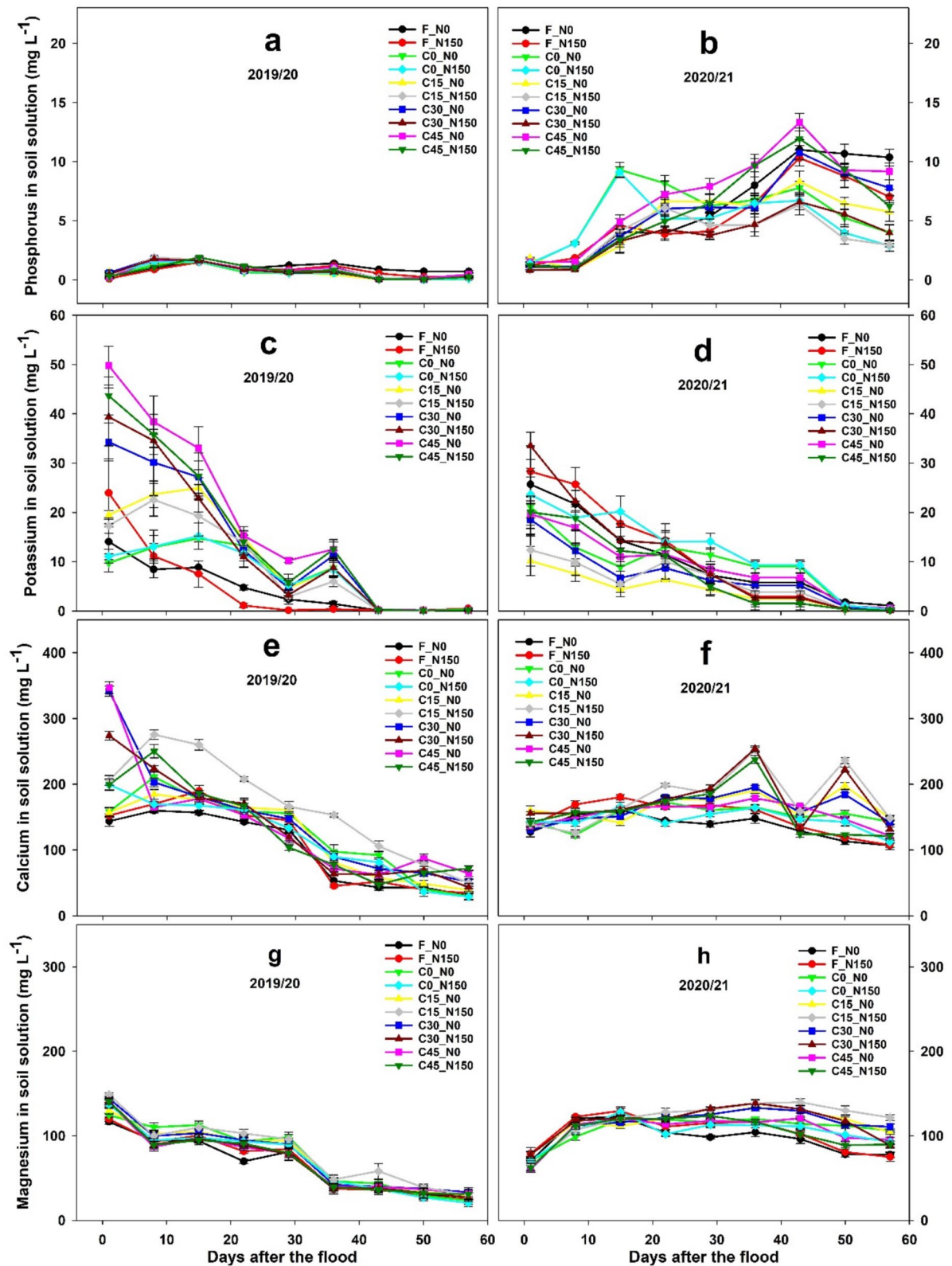
Calcium concentrations in the soil solution were only slightly affected by the treatments. In 2019/2020, descriptive temporal patterns indicated that treatments C30_N0 and C45_N0 showed higher Ca concentrations at 1 day after flooding (Fig. 4e). Between 8 and 43 days after flooding, treatment C15_N150 presented higher Ca concentrations, while the other treatments remained at similar levels (Fig. 4e). In the second season, Ca concentrations were similar among treatments in most sampling events (Fig. 4f). When averaged over the first 29 days after flooding and analyzed statistically, Ca concentrations differed among treatments within each crop season (Table 2).

cultivation; C0—termination of Persian clover at rice sowing; C15—termination of Persian clover 15 days before rice sowing; C30—termination of Persian clover 30 days before rice sowing and C45—termination of Persian clover 45 days before rice sowing

Magnesium concentrations in the soil solution showed similar temporal patterns among treatments within each crop seasons, and this consistency was observed in both seasons (Fig. 4g and h). When averaged over the first 29 days after flooding and analyzed statistically, Mg concentrations were higher in treatments receiving 150 kg N ha⁻¹ compared with unfertilized treatments in both years (Table 2).

Dry matter, tillers, height and physiological indices of irrigated rice

Dry matter accumulation per pot was significantly affected by the treatments. In 2019/2020, the highest values were observed in C0_N0, C0_N150, and C15_N150, whereas in 2020/2021 the greatest accumulation occurred in C0_N150 and C15_N150 (Table 3). Plant height was also influenced by N fertilization. In 2019/2020, the tallest plants were recorded in all clover treatments receiving 150 kg N ha⁻¹, as well as in C0_N0 and C15_N0. In 2020/2021, however, only clover treatments with 150 kg N ha⁻¹ maintained the highest plant height (Table 3). A similar pattern was observed for tiller production: in 2019/2020, higher tiller numbers were found in C0_N0, C0_N150, and C15_N150, while in 2020/2021 the maximum



◀**Fig. 4** Phosphorus concentration in the soil solution in the 2019/20 (a) and 2020/21 (b) growing seasons, potassium in the soil solution in the 2019/20 (c) and 2020/21 (d) growing seasons, calcium in the soil solution in the 2019/20 (e) and 2020/21 (f) growing seasons, and magnesium in the soil solution in the 2019/20 (g) and 2020/21 (h) growing seasons, as a function of different Persian clover termination periods with and without nitrogen application, during the flooding period

number of tillers occurred exclusively in C0_N150 (Table 3).

Physiological indices of irrigated rice responded to both Persian clover termination timing and N fertilization, with consistent effects on plant N status across seasons. Higher chlorophyll and nitrogen balance indices were observed in treatments where Persian clover was terminated closer to rice sowing, particularly under N fertilization, whereas fallow treatments showed lower values (Table S3). Flavonoid and anthocyanin indices varied among treatments and seasons, indicating differences in plant stress and secondary metabolism, but these responses were less consistent than those observed for chlorophyll and nitrogen balance. Detailed values are presented in the Supplementary Material (Table S3).

Irrigated rice dry matter nutrients

Macronutrient accumulation per pot in irrigated rice DM was significantly influenced by Persian clover management and N fertilization (Table 4). Nitrogen accumulation showed strong treatment effects, with C0_N150 consistently presenting the highest values in both crop seasons, which was associated with both greater rice dry matter production and relatively higher N concentration in the biomass (Table S2). In contrast, Fallow_N0 exhibited the lowest N accumulation, reflecting its reduced biomass and nutrient concentration. Potassium accumulation varied between seasons, in 2019/2020, the maximum was observed in C45_N150, coinciding with higher K concentration in rice dry matter under this treatment, whereas Fallow_N0 showed the lowest accumulation. In 2020/2021, the highest K accumulation was observed in C0_N150 and C45_N150, mainly driven by increased biomass, as K concentrations were relatively similar among treatments (Table S2).

Phosphorus accumulation was greatest in C0_N150 during 2019/2020 and in C15_N150 in 2020/2021, while Fallow_N0 maintained the lowest

levels in both years. Calcium accumulation in rice DM reached its maximum in C0_N0 in 2019/2020 and in C0_N150 in 2020/2021, contrasting with the consistently lowest values in Fallow_N0. For Mg, the highest accumulation in 2019/2020 occurred in C0_N0, C0_N150, and C15_N150, while in 2020/2021, C0_N150 and C15_N150 showed the greatest values (Table 4). In both seasons, Fallow_N0 presented the lowest Ca and Mg accumulation, due to the lower biomass, since the concentration was similar to the other treatments (Table S2).

Correlation analysis

Pearson correlation analysis among clover dry matter, soil solution variables, and rice growth indices is presented in the Supplementary Material (Fig. S2). Clover dry matter showed a strong positive correlation with DOC in the soil solution ($r=0.71$). In addition, DOC was positively correlated with rice dry matter production ($r=0.86$), while N-ammonium concentration was positively associated with tiller number ($r=0.74$). Rice dry matter production was also strongly correlated with plant height ($r=0.96$), indicating close relationships between soil nutrient dynamics and rice vegetative growth.

Discussion

Dry matter production

The greater dry matter production of Persian clover in the treatments with termination dates closer to rice sowing (C0, C15 and C30) can be attributed to the longer growth period available for biomass accumulation, compared with the early chemical management at C45 (Peoples et al. 2009; Jensen et al. 2010). A longer vegetative cycle favors enhanced biological nitrogen fixation (BNF) and a greater potential for nutrient cycling, which positively influences the development of subsequent crops in the succession system (Peoples et al. 2009; Jensen et al. 2010). In addition, early chemical terminations (C45 and C30) interrupted clover growth during its main vegetative phase, which typically occurs between June and December in southern Brazil (Bortolini et al. 2012), thereby reducing biomass accumulation mainly by shortening the effective growth period rather

Table 3 Dry matter, height and tillers of irrigated rice, at rates of 0 and 150 kg N ha⁻¹ cultivated in a Planosol in the 2019/2020 and 2020/2021 crop seasons in a greenhouse under different times of chemical management of Persian clover: Fallow – without Persian clover cultivation; C0—termination of

Persian clover at rice sowing; C15—termination of Persian clover 15 days before rice sowing; C30—termination of Persian clover 30 days before rice sowing and C45—termination of Persian clover 45 days before rice sowing

Treatments		Dry matter rice		Height		Tiller	
		Crop season					
		19/20	20/21	19/20	20/21	19/20	20/21
		g pot ⁻¹		cm		Number	
0N	Fallow	43 d	15 f	50 d	42 d	30 e	15 g
	C0_N0	107 a	42 c	60 ab	48 bc	61 ab	51 b
	C15_N0	89 b	38 cd	60 ab	48 bc	50 cd	36 d
	C30_N0	73 c	26 e	55 c	45 cd	44 d	27 e
	C45_N0	72 c	22 e	56 bc	44 d	44 d	22 f
150N	Fallow	73 c	36 d	55 c	45 cd	46 d	36 d
	C0_N150	117 a	61 a	63 a	52 a	66 a	60 a
	C15_N150	109 a	57 a	62 a	51 ab	58 abc	55 b
	C30_N150	91 b	49 b	59 ab	50 ab	51 cd	46 c
	C45_N150	93 b	49 b	61 a	49 ab	56 bc	44 c

Means followed by the same letter within each crop season do not differ from each other according to Tukey's test ($p < 0.05$). Ns: no significant difference

Table 4 Nutrients in the aerial part of irrigated rice, grown at rates of 0 and 150 kg N ha⁻¹ in a Planosol in the 2019/2020 and 2020/2021 crop seasons, in a greenhouse under different times of chemical management: Fallow—without Persian clover cultivation; C0—termination of Persian clover at rice sow-

ing; C15—termination of Persian clover 15 days before rice sowing; C30— termination of Persian clover 30 days before rice sowing and C45—termination of Persian clover 45 days before rice sowing

Treatments		Nitrogen		Potassium		Phosphorus		Calcium		Magnesium	
		Crop Season									
		19/20	20/21	19/20	20/21	19/20	20/21	19/20	20/21	19/20	20/21
		mg pot ⁻¹									
0N	Fallow	414 g	130 j	676 f	394 g	144 f	41 e	126 d	1353 i	95 d	32 g
	C0_N0	1200 b	556 d	946 cd	798 cd	320 b	111 c	417 a	3966 d	318 a	121 c
	C15_N0	968 d	338 g	1005 b	785 d	268 d	101 c	331 b	3279 f	239 b	96 d
	C30_N0	687 ef	251 h	909 d	627 e	207 e	74 d	252 c	2266 g	210 bc	70 e
	C45_N0	676 f	219 i	949 cd	557 f	204 e	65 d	243 c	1975 h	201 c	54 f
150N	Fallow	745 e	394 f	783 e	817 c	217 e	105 c	271 c	3407 e	223 bc	106 d
	C0_N150	1374 a	880 a	792 e	928 a	314 b	174 a	339 b	5289 a	299 a	179 a
	C15_N150	1236 b	714 b	992 b	899 b	364 a	149 b	358 b	5070 b	305 a	166 a
	C30_N150	1004 cd	583 c	967 bc	892 b	286 c	145 b	241 c	4442 c	222 bc	142 b
	C45_N150	1035 c	452 e	1093 a	907 ab	285 c	150 b	256 c	4357 c	231 bc	139 b

Means followed by the same letter within each crop season do not differ from each other according to Tukey's test ($p < 0.05$). Ns: no significant difference

than by exposing the crop to less favorable growing conditions.

Multi-year field evidence indicates that Persian clover terminated near rice sowing can produce high dry matter production (5–6 Mg ha⁻¹), confirming its potential as an effective winter cover crop (Weinert et al. 2023). However, the present study was conducted under controlled conditions, and biomass responses may differ in varying field environments. Such increased biomass production is directly linked to greater nutrient accumulation, since clover residues are an important source not only of N but also of P, K, Ca, and Mg, which are gradually released into the soil solution during decomposition and mineralization processes (Cassman et al. 2002; Xavier et al. 2017). This nutrient release contributes to soil fertility and improves the nutrient supply for rice establishment, especially in systems with reduced dependence on mineral fertilizers (Cassman et al. 2002; Xavier et al. 2017). Therefore, the persistence of Persian clover until sowing maximizes biomass production and, consequently, nutrient returns to the soil, mainly through greater biomass production rather than changes in tissue nutrient concentrations, highlighting its strategic role in integrated crop systems by improving soil quality and reducing the reliance on external nutrient inputs (Weinert et al. 2023). This relationship was supported by Pearson correlation analysis, which showed a strong positive correlation between clover dry matter and DOC in the soil solution ($r=0.71$), indicating that greater residue production contributed to increased carbon availability in the soil solution.

Soil solution

Persian clover provides C to the soil through DM and root exudates, which serve as a C source for microorganisms, while amino acids and proteins present in clover residues provide mineral N to the soil and microorganisms (Thilakarathna et al. 2016; Peoples et al. 2009). This highlights that higher DOC levels in the soil solution may be associated with greater clover biomass accumulation, which was supported by Pearson correlation analysis showing a strong positive relationship between clover dry matter and DOC ($r=0.71$). Furthermore, DOC in the soil solution was strongly correlated with rice dry matter production ($r=0.86$), suggesting that greater carbon availability in the soil solution may contribute to improved

rice growth. Similar results were found by Hu et al. (2024) in a field experiment, with DOC levels 84% higher in plots cultivated with legumes compared to plots under fallow. Clover cultivation, in addition to providing more DOC, contributes to increasing the availability of mineral N, especially N-ammonium, as this N results from the low C:N ratio of legumes, promoting rapid mineralization and availability to plants (Thilakarathna et al. 2016). This greater availability of mineral N was positively associated with rice development, as indicated by the strong correlation between N-ammonium concentration and tiller number ($r=0.74$).

Chemical management carried out close to rice sowing favors greater development of Persian clover plants, increasing their capacity to accumulate potentially mineralizable N-ammonium and make it available in the soil solution (Lucadamo et al. 2022; Weinert et al. 2023). This occurs in clover terminations closer to sowing, such as the C0_N0 treatment. However, in terminations further from sowing, such as C30 and C45, rapid residue decomposition may lead to asynchrony between N mineralization and rice N demand, resulting in lower N availability at early crop stages; although this process does not represent a loss of N from the system, it can reduce short-term plant uptake and alter the dynamics of N retention in the soil (Cai et al. 2018; Carlos et al. 2022). These losses have a significant impact in the absence of N application, since N availability is reduced in floodplain soils (Carlos et al. 2022). On the other hand, the concentration of N-nitrate in the soil solution behaved differently than N-ammonium. Although Persian clover in treatments terminated closer to sowing provides a greater amount of N-nitrate in relation to N-ammonium in the soil, its availability is reduced after soil flooding, because N-nitrate undergoes denitrification under anaerobic conditions after flooding (Sousa et al. 2009).

The smaller reduction of soil solution N under the 150N rate across the chemical management timings may be associated with a priming-like effect (Jiang et al. 2021), although this mechanism was not directly evaluated in the present study. Increased N availability from fertilization can stimulate microbial activity and enhance the mineralization of labile SOM, potentially contributing to greater mineral N availability (Jiang et al. 2021). Similar responses have been reported in systems with winter legumes, where

higher mineral N levels have been observed compared with fallow conditions, suggesting an indirect contribution of residue inputs and fertilization to soil N dynamics (Weinert et al. 2023). However, further studies measuring microbial activity and enzyme dynamics are needed to confirm the occurrence of priming under these conditions.

The N supplied by winter legumes or mineral fertilization may have increased P concentrations in the soil solution by stimulating microbial activity and phosphatase-mediated mineralization of organic P, thereby enhancing P availability (Kritzler and Johnson 2010). However, when Persian clover was chemically terminated close to rice sowing under greater accumulated biomass, plants and microbial demand for P was likely higher, which may have temporarily reduced P availability in the soil solution (Vitousek et al. 2010). This pattern is consistent with the inherently high P requirement of legumes due to their biological N fixation capacity, which intensifies P cycling through increased microbial and enzymatic activity, particularly in species with elevated phosphatase activity (Olde Venterink 2011).

Because K is not structurally bound to organic compounds, it presents greater mobility in the soil following crop residue management. The greater availability of K with longer Persian clover termination intervals suggests a slower release of this nutrient from clover residues, allowing it to remain in the soil solution for a longer period. In addition, the high average K concentration observed in the soil solution under fallow with N application may be related to ionic competition processes, since K^+ and NH_4^+ have similar ionic radius and compete for the same adsorption sites in the soil (Feng et al. 2021). As the solid phase and the soil solution tend toward equilibrium, increased NH_4^+ adsorption can promote K^+ desorption, resulting in higher K concentrations in the soil solution (Sousa et al. 2009).

The resorption of Ca and Mg at soil exchange sites interferes with their concentration in the soil solution (Buss et al. 2011), as peaks in Ca and Mg concentrations in the soil solution immediately after flooding have been previously reported in flooded soils (Sousa 2001), consistent with the patterns observed in this study. Although these nutrients are not directly involved in the reduction reactions of flooded soil, the kinetics of the iron and manganese reactions, directly related to reduction reactions, result in the

displacement of Ca and Mg into the soil solution (Ponnamperuma 1977).

Overall, these results show that the presence and management of Persian clover modify nutrient release patterns, affecting not only C and N cycling but also the availability of P, K, Ca, and Mg in the soil solution, under controlled conditions. While these findings highlight the potential of winter legumes to improve nutrient availability and reduce reliance on synthetic fertilizers, future studies should evaluate these responses under diverse field conditions and longer-term rotations to validate their consistency and agronomic relevance in irrigated rice systems.

Physiological indices, height, tillers and dry matter of irrigated rice

The greater availability of N in the soil under the shortest chemical management interval (clover 0) may result in higher chlorophyll and N balance indexes and lower anthocyanin and flavonoid indexes, indicating improved plant N uptake. This response suggests a better synchronization between N release from Persian clover residues and rice N demand, since chlorophyll content is directly related to N uptake (Nasar et al. 2020). This relationship was supported by a positive correlation between N-ammonium concentration and chlorophyll index ($r=0.47$). In the post-clover system, mineral N availability in the soil solution was more continuous throughout the rice cycle, which likely enhanced N uptake, photosynthetic activity, and photoassimilates production, resulting in increased tillering, plant height, and biomass accumulation (Pasa et al. 2024). Indeed, rice dry matter production showed strong positive correlations with plant height ($r=0.96$) and tiller number ($r=0.79$), indicating that vegetative growth was closely linked to biomass accumulation. Similar patterns were reported by Weinert et al. (2023), who observed sustained mineral N availability during the irrigated rice cycle when Persian clover replaced fallow. Such synchronization between N supply and plant demand reduces the risk of N losses and contributes to improved crop performance. The proximity of Persian clover termination to rice sowing therefore promotes more efficient N use and greater biomass production, primarily due to the high content of amino acids, proteins, and other labile organic compounds in legume residues that accelerate mineralization and nutrient

availability during rice establishment (Peoples et al. 2009; Jensen et al. 2020).

Although P, K, Ca, and Mg accumulation varied among treatments, their patterns did not translate into proportional changes in rice growth variables. This suggests that, under the experimental conditions, these nutrients were not limiting and likely played a secondary role compared to N, which is widely recognized as the primary limiting nutrient in irrigated rice systems (SOSBAI 2022). Thus, while Persian clover influenced the dynamics of multiple nutrients in the soil–plant system, the growth response of rice was primarily driven by improved N synchronization rather than by changes in other macronutrients.

Despite its benefits for nutrient cycling, potential constraints must be considered when adopting late chemical termination under field conditions. One limitation is the physical barrier created by the large amounts of biomass (5–6 Mg ha⁻¹), which can hinder proper seed placement and seedling emergence (McKenzie-Gopsill and Farooque 2023). In addition, residue decomposition may lead to the accumulation of organic acids (e.g., acetic and propionic acids) under poorly drained or excessively wet conditions, impairing early rice development through hypoxia-induced stress (Sousa 2001; Ponnampereuma 1977). These risks emphasize the importance of site-specific management strategies that balance the agronomic benefits of improved N cycling with the operational challenges of residue management in flooded rice systems.

Conclusion

Chemical management of Persian clover at the time of rice sowing markedly increased autumn–winter dry matter production, resulting in 132% greater biomass compared with termination performed 45 days before sowing. Terminating winter legumes closer to sowing increased N availability in the soil solution, which was reflected in higher chlorophyll indices and greater N uptake by the subsequent irrigated rice crop, regardless of the N fertilization rate. In contrast, longer termination intervals tended to increase phosphorus and potassium concentrations in the soil solution during rice vegetative development, indicating that clover management timing plays a key role in regulating nutrient dynamics in flooded rice systems.

This study was conducted under controlled conditions, which may not fully capture the variability of field environments, such as soil heterogeneity, weather fluctuations, and management constraints. Future research should evaluate the effects of Persian clover chemical management under diverse field conditions and longer-term rotations to confirm the observed benefits and optimize nitrogen and nutrient management strategies for irrigated rice systems.

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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

Conflict of interest The authors declare no competing interests.

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