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Introduction of summer cover crops in soft wheat (*Triticum aestivum* spp. *aestivum*) monoculture rotation: effects on agronomic traits and soil properties after three years

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Context: Continuous wheat systems are common across Mediterranean Europe, yet management strategies that simultaneously sustain yield and improve soil health remain poorly defined.

Objective: This study aimed to assess the short-term effects of introducing summer cover crops and different nitrogen rates in a soft wheat monoculture during the system's initial transition toward a new equilibrium.

Methods: A three-year field experiment in northern Italy evaluated the combined effects of summer cover crop species and N fertilization rates on wheat yield, grain protein concentration, nitrogen use efficiency (NUE), and soil organic carbon (SOC) dynamics. Five ground-cover treatments (bare fallow, natural grassing, sorghum, sorghum + cowpea, cowpea + niger) were tested under three nitrogen (N) fertilizer rates (0, 90, 150 kg N ha⁻¹).

Results: Wheat yield and protein were primarily determined by N rate and cover crop residue quality. Legume-based covers (cowpea + niger and sorghum + cowpea) maintained yields comparable to the fertilized fallow at 90 kg N ha⁻¹, while pure sorghum residue required 150 kg N ha⁻¹ to partially offset yield reductions associated with its high C/N ratio. Across all years, NUE declined at 150 kg N ha⁻¹, indicating limited efficiency gains from higher fertilization. Grain protein concentration followed a similar pattern, increasing with N rate but constrained under sorghum cover. Soil analyses showed that cover crops increased total SOC by approximately 6 Mg C ha⁻¹ compared to fallow, with 74% of the additional C stabilized in the mineral-associated organic matter (MAOM) fraction. SOC gains were greatest under sorghum residues but required higher N inputs to sustain yield and grain quality.

Conclusions: These results demonstrate that adjusting N fertilization according to cover crop residue quality is critical to balancing productivity and soil C sequestration in continuous wheat systems. Legume-based summer covers combined with moderate N inputs (90 kg N ha⁻¹) optimized NUE while maintaining yield and enhancing SOC.

Implications: This research provides practical, field-based guidance for integrating cover crops into Mediterranean cereal systems to improve both agronomic performance and soil resource sustainability.

KEYWORDS

grasses, legumes, micronutrients, nitrogen use efficiency, organic matter, sequestration

1 Introduction

The need for an ecological transition in the agri-food sector to make Europe a climate-neutral continent by 2050 (Schebesta et al., 2020) requires developing resilient farming systems that increase soil carbon (C) and reduce reliance on mineral fertilizers, thereby supporting climate change mitigation (Lal, 2001). In this context, cover crops (CC) could play an important role in preserving and restoring soil fertility and mitigating climate change effects.

Cover crops are defined as crops grown primarily for the purpose of protecting and improving soil between periods of regular crop production (Schoolman and Arbuckle, 2022). Cover crops are grown in the bare fallow between cash crops, using residual soil nutrients, and terminated before competing with the following cash crop, either prior to or shortly after sowing (Quintarelli et al., 2022). Cover-cropping adoption is rising rapidly. Between 2017 and 2022, the U.S. cropland area planted with CC increased by 17%, from 6.22 to 7.28 million hectares (Bowman and Morales, 2024). There has been a significant increase in CC also in the European Union (EU), from 6.5 to 8.9% of arable land (Borrelli and Panagos, 2020), and, although it is still underutilized (Kathage et al., 2022), predictions assume a likely significant increase in the future (Fendrich et al., 2023).

Cover crops deliver ecosystem services that improve soil fertility and health. They contribute over time to the buildup of soil organic matter (SOM), enhance soil structure, and improve aggregate stability, essential for nutrient retention and water holding capacity (Villamil et al., 2006; Abdollahi et al., 2014). However, SOM accumulation generally requires several years of consistent CC use, as early effects are most evident in labile SOM fractions rather than bulk soil C (Blanco-Canqui, 2022). In the short term, CC primarily contributes to feeding the fresh, unprotected particulate residues while mineral-associated organic matter represents a slower-turnover pool important for long-term C sequestration. Isolating these SOM pools enables the detection of subtle early changes that may not be apparent when examining total C alone (Kauer et al., 2021). Beyond C dynamics, CC can influence soil nutrient cycling by increasing nitrogen (N) input with N-fixing species, scavenging residual nutrients, reducing leaching losses, and returning nutrients through residue mineralization (Scavo et al., 2022). They may also influence soil concentrations of potentially toxic elements (PTEs) by phytoextracting or redistributing elements such as cadmium (Cd) and nickel (Ni), thereby altering their availability and potential risk in the soil-plant system (Sharma et al., 2025).

Despite the growing interest in CC, in temperate crop areas the research has mainly focused on winter CC, sown after a spring-

summer cash crop, while the number of studies on summer CC in winter wheat (*Triticum aestivum* spp. *aestivum*) cropping system remains limited, with contrasting effects on subsequent wheat systems. Within the available literature, long-term evidence shows that CC can have different effects on wheat yield and overall agronomic performance, yet responses are highly context-dependent, varying with species composition, termination timing, and the synchronization of nutrient release with crop demand (Soares et al., 2023; Büchi et al., 2018). Furthermore, only a few studies investigate the effect on soil and wheat quality and quantity of introducing summer intercropping in the first years in a cereal system based on wheat monoculture (Mubvumba et al., 2021; Nielsen et al., 2016; Wang et al., 2024). Studying cover crop functional type is also relevant. For example, legume-based CC can improve wheat yield by fixing atmospheric N and supplying readily mineralizable residues (Regassa et al., 2023). Conversely, high C/N ratio species, such as grasses, may stimulate microbial N immobilization if decomposition is not aligned with crop needs, limiting N availability to wheat during critical growth stages (Kandel et al., 2020). In regions with low precipitation, water competition can amplify these drawbacks (Singh et al., 2023). Moreover, excessive biomass from CC may hinder wheat establishment, especially if residue management is not optimal (McGuire et al., 1998). These complexities highlight the importance of aligning CC management with the environmental and agronomic conditions of the target region, also considering the agronomic practice, such as N fertilization. Nitrogen inputs can offset immobilization or low residual N, but their efficiency hinges on dose and timing. Results suggest that moderate N applications may optimize nitrogen use efficiency (NUE) in systems with CC (Habbib et al., 2017). However, on the one hand, low N application, especially following CC with high C/N, may result in high microbial N immobilization and limited decomposition of CC residues; on the other hand, excessive N application, especially without synchronization with crop uptake, can result in increased nitrate leaching and nitrous oxide emissions, undermining the environmental benefits of CC (Kumar and Bordoloi, 2024). Consequently, an integrated approach that considers both the type of CC and the N fertilization strategy is essential for balancing productivity, environmental sustainability, and wheat quality, mainly related to the grain protein content. However, the latter has been poorly studied, in particular for wheat grown within a supply chain contract, where the economic profitability is strictly linked also to the achievement of quality requirements (Blandino et al., 2016).

In agricultural areas characterized by low rainfall in the summer period and low availability of water for irrigation, monoculture of

winter cereals is quite common (Adil et al., 2022) and weed control in the intercrop period is difficult and is often managed with the application of total herbicides (e.g., glyphosate) in order to reduce seedbed tillage of the next crop cycle and reduce costs. However, the permissibility of glyphosate use in the future is in strong doubt and is often already limited in food supply chains (Metcalf et al., 2024; Ye et al., 2021). Weed suppression in the intercrop period is another key benefit of CC, particularly when fast-growing, competitive species are used. Mixtures tend to be more effective than monocultures in reducing weed biomass, owing to diverse canopy architectures and growth habits that shade the soil and prevent weed emergence (Wojciechowski et al., 2016; Büchi et al., 2018). However, weed suppression depends on the ability of CC to produce sufficient biomass before termination. If CC establishment is poor, either due to drought or suboptimal seeding timing, weed suppression benefits may be negligible. Mixed-species CC (e.g., grass-legume mixture) may offer synergistic benefits for weed suppression and SOM accumulation but show variable outcomes on wheat yield and NUE (Soares et al., 2023). Furthermore, interactions with N fertilization rate and incorporation timing remain poorly integrated in experimental designs (Habbib et al., 2017), limiting the qualitative and quantitative assessment of effects on the subsequent cash crop, especially in the first few years after CC introduction in a wheat monoculture.

Compared to previously reported research, this study aims to evaluate the effects of introducing CC in the first three years within a highly productive cereal system based on soft wheat monoculture, analyzing the behavior of different types of CC, their effect on soil characteristics (SOM pools, macro-, meso-, and micronutrient contents, and PTE occurrence), and the qualitative and quantitative parameters of wheat in succession. Specifically, we hypothesized that: (i) CC will enhance free-unprotected and mineral-associated organic matter pools, with species effects on C and N contributions; (ii) the introduction of legumes in grass-based CC could mitigate the risk of microbial N immobilization and further enhance organic matter soil pools; (iii) CC may reduce wheat yield and quality unless paired with sufficient N inputs. To test these hypotheses, we investigated the interacting effects of summer CC and N fertilization rate, in the first three years of introduction within a cereal system based on wheat monoculture. Specifically, we aimed to assess the effect of summer CC species, alone or in mixture, on the qualitative and quantitative parameters of the successional cash crop as a function of biomass input and soil adaptation to CC introduction.

2 Material and methods

2.1 Experimental site and treatments

A field trial lasting 3 crop years (2020–21, 2021–22, 2022–23) was conducted in Villafranca Piemonte (TO, Piedmont, Italy). The site has a humid temperate climate with hot summer (Cfa) according to Köppen-Geiger climate classification (Kottek et al., 2006). Daily

temperatures and precipitation were measured at an on-site weather station, from which growing degree days (GDD) and potential evapotranspiration (ET_0) were determined. GDD were obtained as the sum of degrees above 0 °C from the daily mean temperatures. ET_0 was calculated according to the Hargreaves method (Hargreaves and Samani, 1982).

The experiment involved the comparison of different summer CC as intercrops with soft wheat, resulting in 5 ground cover treatments. The compared CC were: S, sudan sorghum (*Sorghum x drummondii*) (cv. Sudal, Mas Seeds Italia, San Pietro di Morubio, VR, Italy); SV, mixture of sudan sorghum and cowpea (*Vigna unguiculata*) (cv. White sounders, MAS Seeds Italia, San Pietro di Morubio, VR, Italy); NV, mixture of cowpea and niger (*Guizotia abyssinica*) (MAS Seeds Italia, San Pietro di Morubio, VR, Italy). Moreover, for each intercrop period, the experiment included a control left to natural grassing by wild flora (NT) and bare soil control (T), in which glyphosate was applied to control weeds during the intercrop period. In each crop year, CC were sown shortly after wheat harvest and straw removal (mid-July). The selection of CC for comparison was based on summarizing the main characteristics of the different types of CC that could be implemented in the agricultural area under study. Sorghum would maximize biomass production (with a high C/N ratio) during the intercrop period due to its physiological C4 mechanism and ability to absorb nutrients from the active soil layer thanks to its fasciculate root system. It is also a well-known species in the Po Valley for use as both fodder and a substrate for biogas digesters. Sorghum + Cowpea would provide the benefits of sorghum biomass production, as well as nitrogen fixation by cowpea, which would mitigate the C/N ratio. Additionally, its taproot system would allow for greater soil exploration depth. Finally, Niger + Cowpea would have provided the benefits of cowpea associated with a species characterized by low C/N ratio tissues and fairly tolerant to water stress.

The seedbed was prepared by crossing a harrow over the wheat stubble. Each CC was sown in 12-cm-wide rows and using a mechanical seeder at a seed rate of 45 kg ha⁻¹ (standard dose of Sorghum in the area and adapted to other treatments based on experience gained in previous studies). In treatments that included mixtures (Sorghum + Cowpea and Niger + Cowpea), species were mixed at 50% plant/plant. In early October, CC were terminated by shredding the biomass and, after one week of natural drying on the soil surface, they were incorporated into the soil by plowing (30 cm deep). The dates of CC sowing and shredding are reported in [Supplementary Table 1](#). The ground cover treatments were kept in fixed positions each year, and plot dimension was 7 x 30 m, with 4 replications for each ground cover treatment.

For wheat sown in successive crop years, a factorial experimental design was set up to compare the 5 ground cover treatments (bare soil control, natural grassing, Sorghum, Sorghum + Cowpea, and Niger + Cowpea) of the intercrop period and 3 levels of N fertilization (N rate) (0, 90, 150 kg N ha⁻¹) applied during wheat growing season. The treatments were assigned to experimental units using a split plot design, with the ground cover in the intercrop period as main factor and wheat N fertilization as the sub-factor with four replicates; the sub-plots were 6.5 x 2 m and were maintained in the same position

within the field for the 3 growing seasons. Standard local agronomic practices were followed. Briefly, the wheat genotype used was Bologna (SIS, San Lazzaro di Sabena, Bologna, BO, Italy), a high protein wheat cultivar widely grown in the growing area and the reference cultivar in the contract with food industries (Blandino et al., 2016). The wheat seedbed was prepared by a power harrow over the plowed soil. Wheat was sown in October – November in 12-cm-wide rows and using a mechanical seeder at seed density of 450 seeds m⁻².

Ammonium nitrate (33.5% N) was used as fertilizer and N was applied for each N rate split at the tillering stage (GS 23) and at the stem elongation stage (GS 31). Based on soil analysis (Supplementary Table 2), no phosphate or potash fertilizers were applied in any of the growing seasons. Chemical weed control in wheat was carried out with fluroxypyr (57.6 g L⁻¹) + clopyralid (26.4 g L⁻¹) + MCPA (238.1 g L⁻¹) (ArianeTM II, Corteva Agriscience Italia s.r.l., Cremona, Italy) from late tillering to mid stem elongation and at the maximum labeled dose (4 L ha⁻¹). To control foliar and spike diseases, the plots were treated with a mixture of benzovindiflupyr (75 g L⁻¹) + prothioconazole (150 g L⁻¹) (Elatus[®] Era, Syngenta Italia S.p.A., Milano, Italy) at stem elongation (GS 33) and with the mixture of triazole fungicide prothioconazole (125 g L⁻¹) and tebuconazole (125 g L⁻¹) (Prosaro[®], Bayer Cropscience, Milan, Italy) at flowering (GS 62). The dates of sowing, fertilization, spraying and harvest are given in Supplementary Table 1.

The experiment was conducted in loam soil (43.4% sand, 47.5% silt, 9.1% clay) that had been under continuous wheat cultivation for over a decade. Before the trial, during the summer fallow period, the field was kept bare and weeds were controlled using glyphosate (carried out about 1 month after wheat harvest). The main chemical soil parameters measured at 0–30 cm of depth at the beginning of the experiment are reported in Supplementary Table 2. Briefly, the soil had a neutral reaction, low SOM content, average total N content and a C/N ratio of 7.6. Soil was also characterized by high available phosphorus content, average exchangeable potassium content and medium cation exchange capacity (CEC = 12.4 cmol⁽⁺⁾ kg⁻¹).

2.2 Cover crop analysis

Each season, CC and wild flora biomass was measured just before shredding (October) by collecting aboveground material from 1 m² per plot, drying it at 110 °C for 72 h, and weighing it. A 2 kg subsample per plot was ground (0.5 mm) and analyzed for C and N via elemental analyzer, and for phosphorus (P) and potassium (K) by acid digestion followed by inductively coupled plasma optical emission spectroscopy (ICP-OES). For each macronutrient the CC uptake was obtained by multiplying the percent element content by the dry biomass yield per hectare. During CC biomass sampling, the occurrence of weed species was verified by counting the number of plants per surface unit (Supplementary Table 3).

2.3 Soil organic matter fractionation

SOM fractionation was conducted at the end of the experiment, following wheat harvest in July 2023. A composite soil sample was

collected from each plot at five different sampling points, at a depth of 0–30 cm, corresponding to the soil layer in which the biomass produced by the CC was incorporated through plowing. Bulk density was measured at the time of soil sampling and assumed constant for SOM stock calculations. Samples were air-dried and gently sieved (2 mm) to remove large particles while preserving aggregate structure. SOM was fractionated into three distinct components using a modified density separation method based on Cerli et al. (2012). The resulting components were as follows: (1) free POM (fPOM), which is unbound or minimally associated with minerals; (2) occluded POM (oPOM), released from soil aggregates through sonication; and (3) mineral-associated organic matter (MAOM), tightly bound to soil minerals.

Twenty-five grams of soil were submerged in 125 mL of sodium polytungstate (NaPT) solution (1.6 g cm⁻³) in a 250 mL centrifuge bottle. The suspension was gently mixed to ensure full wetting while maintaining aggregate integrity. After a 30-minute settling period, the mixture was centrifuged at 8000 rpm for 40 minutes. The supernatant, containing the fPOM, was decanted through a 0.7-µm GF/F filter and rinsed with deionized water until the filtrate conductivity dropped below 20 µS cm⁻¹. The fPOM was then oven-dried at 40 °C. The residual fraction underwent ultrasonic treatment (Sonoplus HD 2200, Bandelin electronic GmbH & Co. KG, Berlin, Germany) with an energy input of 275 J mL⁻¹ to release oPOM without dispersing clay particles. Sonication was performed in an ice bath to maintain the sample temperature below 40 °C. The suspension was then centrifuged at 10,000 rpm for 40 minutes, and the floating oPOM was recovered, rinsed with deionized water, and oven-dried at 40 °C. After removing oPOM, the remaining fraction was washed repeatedly with deionized water to extract residual NaPT. Washing cycles included centrifugation at 10,000 rpm for at least 30 minutes, until the solution conductivity was below 50 µS cm⁻¹. The sediment, representing MAOM, was oven-dried at 40 °C.

Dried bulk soil and OM fractions were homogenized using a mortar and pestle. C and N contents were quantified through dry combustion using UNICUBE elemental analyzer (Elementar GmbH, Germany). The stock of total soil organic C, total N, and individual OM fractions was calculated by multiplying the concentration of organic C or N (g kg⁻¹ soil) by the soil bulk density (g cm⁻³) and the depth of the soil sample (cm).

2.4 Wheat crop measurements and analysis

In each growing season, a hand-held optical sensing device, RapidSCAN[®] CS-45 (Holland Scientific, Lincoln, NE, USA), was used to measure the Normalized Difference Vegetation Index (NDVI) throughout the whole wheat crop cycle. The instrument was held approximately 160 cm above ground, and its effective spatial resolution was 1 m for the full length of the plot (6.5 m). This assessment was performed approximately every 7 days from leaf emergence to full senescence. The NDVI values were proportional to the crop biomass and greenness (Blandino et al., 2020). The Area Under Canopy Greenness Curve (AUCGC) was calculated during the crop cycle for each treatment from the NDVI measurement for each observation date and using Equation 1 (Blandino et al., 2020):

$$AUCGC = \sum_i^{n-1} \left\{ \left[\frac{(R_i + R_{i+1})}{2} \right] (t_{i+1} - t_i) \right\} \quad (1)$$

where R is the NDVI value, t is the time of observation and n is the number of observations.

Starting with the second crop year, the number of ears per unit of grown area (spike density) was counted manually before the harvest in two sampling areas (625 cm²) per plot. The number of kernels per spike was calculated in 10 randomly selected spikes from each sampling area (Sardella et al., 2023). Plant height was measured on 20 randomly selected plants (soil to spike tip). The plots were harvested using a Walter Wintersteiger cereal plot combine harvester. Grain moisture and the test weight (TW) were analyzed using a Dickey-John GAC2100 grain analyzer (Dickey-John Corp., Auburn, IL, USA). Grain yield results at harvest moisture were converted to dry matter.

The harvested grains were mixed thoroughly, and representative sub-samples (500 g) were ground to whole-meal using a laboratory centrifugal mill equipped with a 1 mm sieve (Model ZM-200, Retsch, Haan, Germany). The grain protein content (N * 5.7, dry weight, AACC method 39-10.01) was determined by means of an NIR System Model 6500 (FOSS-NIRSystems, Laurel, MD, US). Thousand kernel weight (TKW) was determined on two 200-kernel sets for each sample using an electronic balance. Only for wheat harvested in 2023, at the end of 3-year experiment, whole meal samples from each plot were further fine-grained with a Cyclotec 1093 Sample Mill (Foss Tecator, Sweden) for the analysis of element content in the grain. Samples were pre-treated through microwave-assisted acid digestion. About 500 mg of each sample was digested with 6 mL of HNO₃ and 2 mL of H₂O₂ using an MLS 1200 Mega microwave (Milestone, Italy). After cooling, the samples were filtered, and the filtrate was diluted to 50 mL with ultra-pure water. Acid extracts were analyzed for macro (P and K), meso (Ca and Mg), micronutrient (Fe, Mn, Cu, Zn) and PTEs (Ni and Cd) content using ICP-OES on an Agilent 5100 (Agilent Technologies, USA) and graphite furnace atomic absorption spectroscopy (GF-AAS) on a PerkinElmer AAnalyst 800 (PerkinElmer, USA).

In line with local practice, straw was harvested and not incorporated into soil. The straw was collected behind the combine-harvester and weighed immediately after harvesting. The harvest index (HI) values were obtained by dividing the grain yield by the aboveground biomass (grain + straw) of each sample, expressed as a percentage (Sardella et al., 2023). The NUE was expressed according to different indices.

Partial-factor Productivity (PFP) was calculated by dividing the grain yield by the amount of N supplied as mineral fertilizer (Congreves et al., 2021), according to Equation 2:

$$PFP = \frac{GY [kg \ ha^{-1}]}{N [kg \ ha^{-1}]} \quad (2)$$

Where GY is the grain yield and N is the nitrogen fertilization rate.

Partial Nutrient Balance (PNB) was calculated by dividing the amount of nitrogen taken by the grain by the amount of N supplied as mineral fertilizer (De Santis et al., 2025) according to Equation 3:

$$PNB = \frac{GY [kg \ ha^{-1}] * (GPC[\%] * 0.1754)}{N [kg \ ha^{-1}]} \quad (3)$$

Where GY is the grain yield, GPC is the grain protein content and N is the nitrogen fertilization rate.

Agronomic efficiency (AE) was calculated dividing the grain yield difference between N fertilized wheat (90 or 150 kg N ha⁻¹) and unfertilized wheat (0 kg N ha⁻¹) in succession to each ground cover treatment by the fertilizer units applied (Congreves et al., 2021) according to Equation 4:

$$AE = \frac{GY_f [kg \ ha^{-1}] - GY_0 [kg \ ha^{-1}]}{N [kg \ ha^{-1}]} \quad (4)$$

Where GY_f is the grain yield of wheat treated with fertilizer (90 or 150 kg N ha⁻¹), GY₀ is the grain yield of wheat without fertilizer (0 kg N ha⁻¹), and N is the nitrogen fertilization rate.

Recovery efficiency (RE) was calculated by dividing the difference between the N removed from the GY of fertilized wheat (90 or 150 kg N ha⁻¹) and unfertilized wheat (0 kg N ha⁻¹) in succession to each ground cover treatment by the fertilizer units applied (Congreves et al., 2021) according to Equation 5:

$$RE = \frac{Nuptake_{(GY_f)} [kg \ ha^{-1}] - Nuptake_{(GY_0)} [kg \ ha^{-1}]}{N [kg \ ha^{-1}]} \quad (5)$$

Where Nuptake_(GY_f) is the grain nitrogen uptake of fertilized wheat (90 or 150 kg N ha⁻¹), calculated as grain yield (kg ha⁻¹) × grain protein concentration × 0.1754; Nuptake_(GY₀) is the grain nitrogen uptake of unfertilized wheat (0 kg N ha⁻¹), calculated using the same procedure; and N is the nitrogen fertilization rate (kg ha⁻¹).

2.5 Statistical analysis

Kolmogorov-Smirnov normality test and Levene's test were performed to check the normal distribution and homogeneity of variances. For CC, analysis of variance (ANOVA) was performed for biomass yield, nutrient concentration and total uptake and weed occurrence with the ground cover treatment and intercrop period as independent factors. Moreover, ANOVA was run separately for each intercrop period, to analyze the effect of ground cover treatments on the previously reported parameters. For wheat, ANOVA was performed on agronomic, yield and qualitative traits with ground cover, N rate, and crop year as independent factors. In addition, ANOVA was run separately for each crop year, to study the effect of ground cover and N and their interaction on agronomic parameters and, only for 2023, the content of macro, meso and micronutrients and potential toxic elements in wheat grains and the SOM fractionation of C, N and C/N on the soil samples. Multiple comparison tests, according to the Ryan-Einot-Gabriel-Welsh F (REGW-F) test, were performed on the treatment means. Statistical analysis of the data was conducted with the SPSS software package, version 24.0.

3 Results

3.1 Weather conditions

The three growing seasons showed distinct weather patterns in rainfall and temperature, resulting in variations in ET_0 and GDD (Table 1). The 2021 intercrop period experienced frequent rainfall and mild temperatures, especially in August. In contrast, 2022 was marked by severe drought and high temperatures. The 2023 intercrop period recorded total rainfall amounts similar to 2021, but with notably warmer temperatures. For wheat crop year, 2020–21 had moderate temperatures and regular rainfall, while 2021–22 was extremely dry and warm. The 2022–23 crop year featured frequent rainfall, coupled with cooler temperatures particularly during the ripening phase (May–June). Additionally, a hailstorm hit the wheat during the ripening stage in May 2023, resulting in a slight grain shelling, which was uniform across the entire field.

3.2 Cover crop biomass and weed infestation

Ground cover significantly influenced biomass production and composition (Table 2). Biomass yield and organic C were significantly higher under sorghum-based CC (Sorghum and Sorghum + Cowpea), showing increases of 95% compared to natural grassing and Niger + Cowpea, which did not differ significantly from each other. Sorghum presence also led to a higher C/N ratio compared to natural grassing and Niger + Cowpea, with no significant differences between Sorghum and Sorghum + Cowpea treatments. Regarding intercrop period, biomass in 2020 was 52% higher compared to 2021 and 2022, accompanied by a 48% increase in organic C, a 20% decrease in the C/N ratio, and a greater macronutrient uptake.

In terms of nutrient concentration (Supplementary Table 4), N, P, and K levels in sorghum-based treatments (Sorghum and Sorghum + Cowpea) were lower than in Niger + Cowpea by 40, 42, and 54%, respectively. However, due to higher biomass production, total N and P uptake (Table 2) over the three intercrop periods was comparable among CC treatments, while K uptake was significantly higher in Niger + Cowpea (+19%). Although natural grassing plots showed higher N and K concentrations (Supplementary Table 4) in weed biomass compared to Sorghum and Sorghum + Cowpea, overall macronutrient uptake was lower in natural grassing compared to CC treatments (Table 2). A significant intercrop period $\times$ ground cover interaction was observed for biomass yield, organic C, and P–K uptake. In 2020, organic C in biomass from sorghum treatments (Sorghum and Sorghum + Cowpea) was 154% higher than in Niger + Cowpea, whereas this difference narrowed to 68% in 2021 and 55% in 2022. Sorghum biomass production remained consistently higher than natural grassing and Niger + Cowpea across all intercrop periods, though the magnitude of difference was greatest in 2020 (+141%), compared to 2021 (+72%) and 2022 (+59%), reflecting more favorable conditions for crop growth in 2020. Nutrient uptake patterns varied by intercrop period. In 2021, PK uptake was significantly higher in Niger + Cowpea, while in 2020,

natural grassing plots had significantly lower PK uptake than CC treatments.

Weed density, influenced by summer rainfall patterns, was significantly higher in 2020 compared to 2021 and 2022. All CC reduced weed growth effectively, achieving an average suppression of 84% in 2020 and 2021 and 90% in 2022. The composition in terms of density and species changed considerably over the three intercrop periods (Supplementary Table 3). 2020 was by far the intercrop period with the highest number of plants per square meter compared to 2021 (+86%) and 2022 (+147%). In all three intercrop periods, the Sorghum + Cowpea mixture reported a species ratio at termination time of 2.33:1 sorghum:cowpea, therefore significantly unbalanced in comparison to the proportion of sowing. The Niger + Cowpea mixture reported an average species ratio of 3.5:1 niger:cowpea in 2020 and in 2021, but in 2022 the ratio is reversed to 1:12 niger:cowpea. The number of weeds is much higher in the natural grassing treatment for all three years (+600% compared to the average of treatments that included CC sowing during the three intercrop period). For all three intercrop period, the most prevalent weed species is Sorghum halepense, C4 perennial grass weed that reproduces by rhizomes and seeds (Liu et al., 2019).

3.3 Soil characteristics

3.3.1 Carbon stocks and soil organic matter fractions

After three years of cultivation, CC treatments significantly increased soil C stocks compared to the bare soil control (Figure 1; Supplementary Table 5). C stock rose from 45 Mg C ha⁻¹ in the bare soil control to an average of 51 MgC ha⁻¹ in the fields with CC applications, representing an increase of 13%. Analysis of SOM fractions revealed significant differences for fPOM and MAOM, while no significant differences were observed for oPOM. Among treatments, natural grassing exhibited the highest fPOM accumulation (+72% compared with treatments that included CC), followed by Sorghum and Sorghum + Cowpea, whereas Niger + Cowpea showed the lowest performance (-30% compared with treatments that included sorghum). Importantly, most of the C accumulation from CC was associated with the MAOM fraction, which is more resistant to degradation and contributes to long-term C sequestration. While N rate application did not significantly alter total C stocks, it affected the distribution of C among SOM fractions. Specifically, mineral N application shifted C allocation from the refractory MAOM fraction to the more labile POM fractions.

3.3.2 Total soil nitrogen and SOM nitrogen fractions

Trends in total soil N closely mirrored those observed for C (Figure 1). Treatments that have planned biomass production during intercrop period (natural grassing, Sorghum, Sorghum + Cowpea, and Niger + Cowpea) generally enhanced soil N accumulation (+9.6% compared with bare soil control), with the natural grassing and Niger + Cowpea treatments achieving the highest increases. In contrast, treatments involving sorghum

TABLE 1 Monthly rainfall, average daily temperature, growing degree days (GDD) and potential evapotranspiration (ET₀) in the 3 growing seasons, considering the intercropping period (IP) and crop year (CY).

Growing season Month	2020-21				2021-22				2022-23			
	Rainfall ¹ (mm)	Temperature ² (°C)	GDD ³ (Σ°C d ⁻¹)	ET ₀ ⁴ (mm)	Rainfall (mm)	Temperature (°C)	GDD (Σ°C d ⁻¹)	ET ₀ (mm)	Rainfall (mm)	Temperature (°C)	GDD (Σ°C d ⁻¹)	ET ₀ (mm)
July	24	23.1	899	184.6	73	22.7	953	180.3	58	25.9	1081	209.3
August	116	22.9	912	164.0	1	22.3	967	166.2	71	23.6	1003	170.7
September	47	18.8	752	108.2	17	19.4	837	113.5	57	18.4	783	107.6
October	77	11.8	524	55.4	24	11.5	859	62.4	37	15.7	707	66.2
November	3	7.4	390	30.7	163	6.6	330	25.0	50	6.7	429	33.5
December	44	2.9	202	16.7	8	0.5	191	17.8	54	1.0	194	16.7
January	114	1.5	218	20.8	0	1.0	271	23.7	11	2.4	261	23.7
February	20	5.4	307	35.7	1	4.6	381	41.7	21	3.9	338	38.6
March	14	8.0	476	74.4	15	6.9	422	67.7	26	8.9	518	79.5
April	51	10.9	529	95.2	27	12.3	610	107.9	47	12.1	592	114.8
May	81	15.7	704	141.9	76	19.2	816	154.2	334 ⁵	15.9	679	125.6
June	66	22.2	892	176.1	49	23.6	969	198.8	69	21.1	839	161.9
IP (July-Sep)	187	21.6	2562	457	92	21.4	2756	460	185	22.7	2866	488
CY (Oct-June)	470	9.5	4240	647	363	9.5	4850	699	648	9.8	4556	661
July-June	657	12.7	6802	1104	455	12.6	7606	1159	833	13.0	7422	1148

¹Summation of daily data.
²Average daily average temperatures.
³Accumulated growing degree days for each experiment using a 0 °C base value.
⁴Summation of daily potential evapotranspiration (Hargreaves' method).
⁵Hail event occurs with wheat at ripening stage.

TABLE 2 Effect of ground cover (GC) management on cover crop biomass, organic carbon (C org), carbon/nitrogen ratio (C/N), nitrogen (N), phosphorus (P) and potassium (K) uptake and weed infestation at the termination time in 3 intercropping periods (IP).

Factor	Source of variation	Biomass (t DM ha ⁻¹)	C org (kg ha ⁻¹)	C/N	Nutrient uptake (kg ha ⁻¹)			Weed density (plant m ⁻²)
					N	P	K	
GC	T	--	--	--	--	--	--	--
	NT	6.0 b	2469 b	27.4 b	99 b	16 b	173 c	123 a
	S	12.8 a	5628 a	43.0 a	136 a	34 a	273 b	13 b
	SV	12.9 a	5605 a	38.6 a	151 a	34 a	276 b	8 b
	NV	7.2 b	2888 b	23.5 b	129 a	32 a	327 a	35 b
	<i>p</i> -value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.009
CY	2020	12.4 a	5208 a	28 b	189 a	44 a	416 a	62 a
	2021	8.1 b	3340 b	35.1 a	93 b	19 c	180 b	35 b
	2022	8.2 b	3681 b	34.3 a	111 b	23 b	184 b	36 b
	<i>p</i> -value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
GC x IP	<i>p</i> -value	<0.001	<0.001	0.123	0.074	<0.001	<0.001	0.039
2020	T	--	--	--	--	--	--	--
	NT	6.6 b	2737 b	21.2 c	144 c	19 b	243 b	168 a
	S	18.7 a	8155 a	40.4 a	202 ab	56 a	470 a	8 b
	SV	17.5 a	7580 a	32.7 b	234 a	51 a	423 a	8 b
	NV	8.3 b	3098 b	17.5 d	177 bc	51 a	540 a	64 b
	<i>p</i> -value	<0.001	<0.001	<0.001	0.007	<0.001	<0.001	<0.001
2021	T	--	--	--	--	--	--	--
	NT	5.4 b	2078 b	27.9 b	79 a	14 b	133 b	94 a
	S	10.0 a	4273 a	43.1 a	100 a	19 ab	144 b	18 b
	SV	10.7 a	4600 a	45.4 a	104 a	19 ab	168 b	9 b
	NV	6.6 b	2640 b	28.6 b	94 a	24 a	268 a	18 b
	<i>p</i> -value	0.001	<0.001	0.003	0.524	0.020	0.002	<0.001
2022	T	--	--	--	--	--	--	--
	NT	6.0 b	2633 b	35.8 ab	73 a	14 b	111 a	103 a
	S	10.5 a	4748 a	45.4 a	107 a	30 a	224 a	12 b
	SV	9.5 a	4311 a	37.6 a	116 a	25 ab	200 a	8 b
	NV	6.6 b	2927 b	22.8 b	128 a	21 ab	173 a	12 b
	<i>p</i> -value	0.003	0.002	0.008	0.056	0.039	0.072	0.001
SEM		0.64	290.51	1.65	8.31	2.35	22.19	8.66

For each factor, values followed by different letters are significantly different. The level of significance (*p*-value) is reported in the table.

Ground Cover: T, bare soil control; NT, natural grassing; S, Sorghum; SV, Sorghum + Cowpea; NV, Niger + Cowpea.

Field experiment carried out in 3 years, expressed as Intercrop Period (IP).

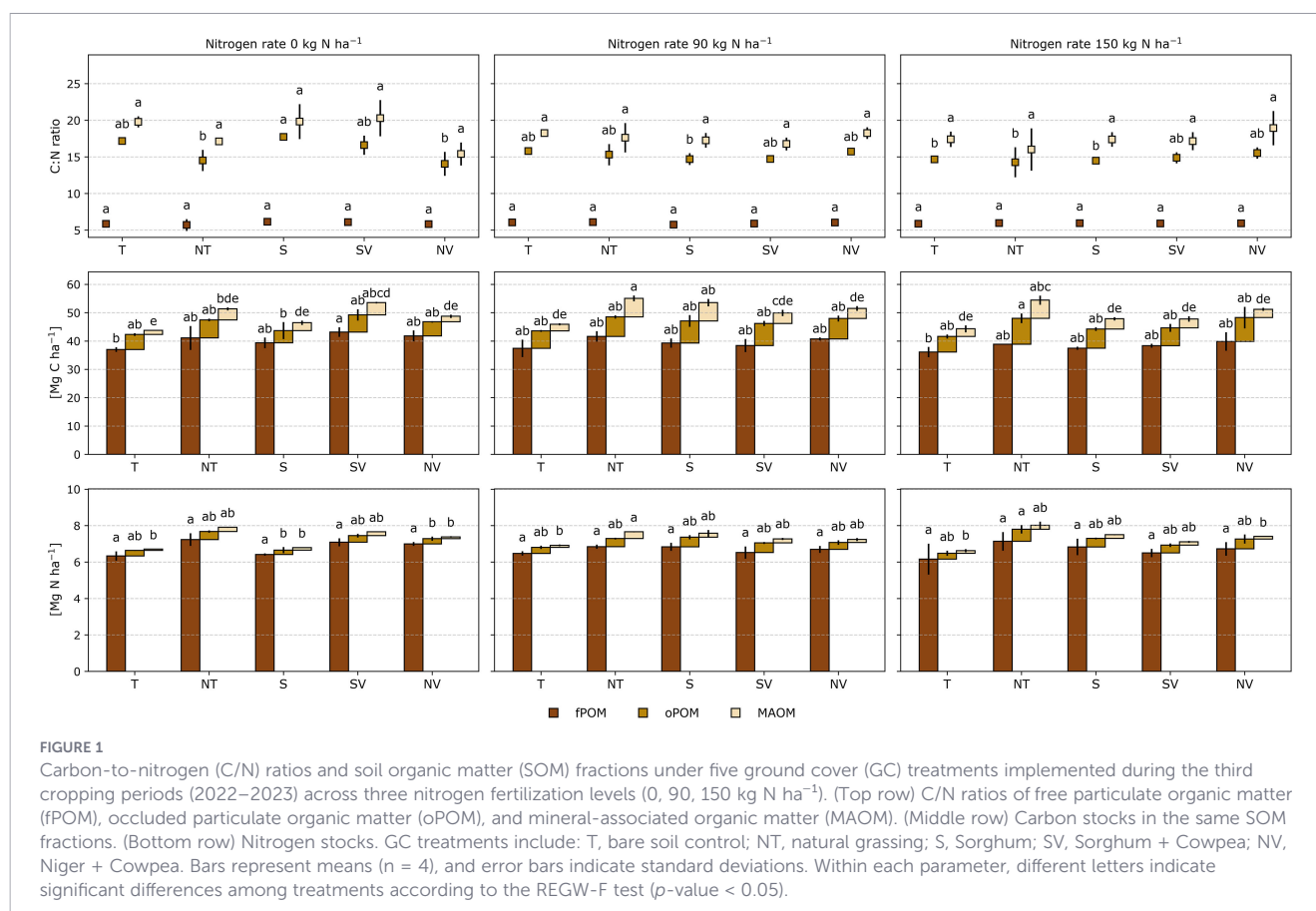
SEM, standard error of means.

(Sorghum and Sorghum + Cowpea) exhibited lower N accumulation (-4.5% compared with natural grassing and Niger + Cowpea).

Analysis of N distribution among SOM fractions showed a doubling of N in the fPOM fraction, increasing from 0.1 Mg N ha⁻¹ in the control to values of 0.2 Mg N ha⁻¹ or more. Additionally, N content in the oPOM fraction increased by 37% in treatments that included biomass production compared with bare soil control. For the MAOM fraction, N levels for treatments that included biomass

production in intercrop period (natural grassing, Sorghum, Sorghum + Cowpea, and Niger + Cowpea) increased by 8% compared with bare soil control.

Regarding the N rate factor, the mineral fertilizer application (90 and 150 kg N ha⁻¹) led to a substantial increase in N within the fPOM and oPOM fractions, by +33% and +39%, respectively, compared with treatment with 0 kg N ha⁻¹. However, no significant changes were observed in the N content of the MAOM fraction.



3.4 Wheat agronomic traits

Ground cover treatments significantly affected wheat agronomic traits (Table 3). Sorghum-based treatments (Sorghum and Sorghum + Cowpea) recorded lower AUCGC, plant height, and spike density compared to other treatments, with reductions of 12, 7.4, and 18%, respectively. Across all ground cover treatments involving biomass production during the intercrop period (natural grassing, Sorghum, Sorghum + Cowpea, and Niger + Cowpea), wheat development was consistently lower than the bare soil control, with AUCGC reduced by 16%, plant height by 10%, straw production by 34%, spike density by 21%, and kernel number per spike by 8%. In contrast, HI and TKW were higher in all ground cover treatments compared to the bare soil control, with increases of 8% and 6%, respectively. Straw yield showed no significant differences among natural grassing, Sorghum, Sorghum + Cowpea, and Niger + Cowpea treatments, which was significantly lower than bare soil control. Specifically, sorghum treatments (Sorghum and Sorghum + Cowpea) showed the greatest reductions: AUCGC decreased by 20%, plant height by 12%, and spike density by 27% compared to bare soil control.

N rate significantly influenced the plant development and several yield components. Plots receiving 150 kg N ha⁻¹ consistently showed higher AUCGC (+12%), plant height (+5%), straw yield (+25%), HI (+8%), spike density (+8%), kernel per spike (+12%) compared to the unfertilized control (0 kg N ha⁻¹). Within yield components, only TKW was unaffected by N rate.

As far as crop year is concerned, AUCGC was significantly influenced by the growing season, decreasing by 10% in 2022 compared to 2021, and by 39.5% in 2023 relative to 2022.

Significant ground cover × N rate and ground cover × crop year interactions were observed for AUCGC and HI (Supplementary Table 6). In 2021, AUCGC was influenced by N rate but not by ground cover. In 2022 and 2023, sorghum treatments (Sorghum and Sorghum + Cowpea) consistently showed the lowest AUCGC values. HI was significantly higher (+11%) in 2022 in plots with CC sowing, while no differences were observed in 2023.

3.5 Wheat grain yield and quality

Grain yield was significantly affected by ground cover treatments (Table 4). On average, yield decreased by 20% when biomass from CC or natural infestation during the intercrop period was incorporated into the soil, compared to the bare soil control. Grass-dominant treatments (natural grassing, Sorghum, and Sorghum + Cowpea) showed a greater reduction (-22%) compared to bare soil control, and a smaller but still significant reduction (-10%) compared to Niger + Cowpea. No significant differences were observed among natural grassing, Sorghum, and Sorghum + Cowpea. N rate positively affected yield: 90 and 150 kg N ha⁻¹ resulted in an increase in wheat productivity of 24% and 48%, respectively.

The interaction between ground cover and N rate was significant: the highest grain yield was reported by the bare soil control with the application of 150 kg N ha⁻¹ (Figure 2); the Niger + Cowpea treatment

TABLE 3 Effect of ground cover (GC) management during intercropping periods and nitrogen fertilization rate (N rate) on AUCGC, plant height, straw yield, Harvest Index (HI), spike density, kernel per spike and thousand kernel weight (TKW) of the wheat in succession in 3 crop years (CY).

Factor	Source of variation	AUCGC (NDVI-day)	Plant height (cm)	Straw yield (t DM ha ⁻¹)	HI	Spike density (n° m ⁻²)	Kernel per spike (n° spike ⁻¹)	TKW (g)
GC	T	91.28 a	81.76 a	8.27 a	43.67 c	816.5 a	39.8 a	30.36 b
	NT	80.84 b	76.00 b	5.76 b	45.10 bc	715.8 b	37.3 b	31.76 a
	S	73.44 d	71.58 c	5.31 b	47.6 ab	594.2 c	35.1 c	32.00 a
	SV	73.40 d	72.08 c	5.11 b	48.85 a	602.7 c	36.1 bc	32.25 a
	NV	77.65 c	74.89 b	5.77 b	48.23 a	662.7 bc	37.8 b	32.18 a
	p-value	<0.001	<0.001	<0.001	0.016	<0.001	<0.001	<0.001
N rate	0	73.27 c	72.78 b	5.21 b	45.53 b	632.8 b	34.3 b	31.52 a
	90	80.48 b	76.62 a	6.29 a	45.36 b	689.6 a	38.4 a	31.67 a
	150	82.33 a	76.20 a	6.51 a	49.23 a	709.3 a	39.1 a	31.92 a
	p-value	<0.001	<0.001	<0.001	<0.001	0.013	<0.001	0.707
CY	2021	101.49 a	- -	- -	- -	- -	- -	35.77 a
	2022	91.38 b	72.82 b	5.43 b	51.08 a	584.5 b	37.0 a	30.19 c
	2023	55.31 c	77.69 a	7.79 a	35.19 b	774.2 a	37.4 a	30.99 b
	p-value	<0.001	<0.001	<0.001	<0.001	<0.001	0.712	<0.001
GC x N	p-value	0.013	0.839	0.945	0.049	0.487	0.067	0.386
GC x CY	p-value	<0.001	0.318	0.491	<0.001	<0.001	0.413	<0.001
N x CY	p-value	<0.001	0.436	<0.001	<0.001	0.067	0.301	0.04
GC x N x CY	p-value	0.108	0.341	0.226	0.300	0.036	0.14	0.004
SEM		1.54	0.57	0.20	0.86	17.2	0.36	0.21

For each factor, values followed by different letters are significantly different. The level of significance (*p*-value) is reported in the table.

Ground Cover: T, bare soil control; NT, natural grassing; S, Sorghum; SV, Sorghum + Cowpea; NV, Niger + Cowpea.

N rate: total nitrogen rate applied to wheat as top dressed ammonium nitrate between tillering and the stem elongation growth stages (0, 90, 150 kg N ha⁻¹).

Field experiment carried out in the 3 Crop Years (CY).

SEM, standard error of means.

with 150 kg N ha⁻¹ reports a 10% lower yield than the previous one, while Sorghum treatment unfertilized (0 kg N ha⁻¹) was the worst productive combination, with a yield gap of 55% in comparison with bare soil control combined with a 150 kg N ha⁻¹ fertilization.

On average, yield declined progressively across considered crop years, although the interactions among all factors with crop year were always significant. The effect of N rate on grain yield was more evident in 2022 and 2023, while in 2021, only the 0 kg N ha⁻¹ treatment differed significantly from fertilized ones. In each crop year, wheat produced significantly more following treatments without sorghum biomass incorporation into the soil (bare soil control) (Supplementary Table 7): in 2021, wheat after Sorghum reported a yield 6% lower than bare soil control, while in subsequent crop year, the production delta reached a reduction of 26% compared to bare soil control.

On average, TW was higher in treatments where a CC was incorporated into the soil (Sorghum, Sorghum + Cowpea, and Niger + Cowpea), while N rate did not affect this qualitative trait. Furthermore, the effect was related to the interaction with year: in 2021 and 2022, the highest N rate resulted in a significant increase and decrease of this qualitative trait, respectively (Supplementary Table 7). Grain protein content was significantly lower in ground cover with CC (Sorghum, Sorghum + Cowpea, and Niger + Cowpea), showing on average a 1.1% decrease compared to bare

soil control and natural grassing, which did not differ from each other. As expected, grain protein content increased with the N rate, showing an average increase of 1.0% (90 kg N ha⁻¹) and 1.8% (150 kg N ha⁻¹) compared to unfertilized control. 2023 had the highest average grain protein content, followed by 2022 and 2021. The ground cover × N rate interaction was significant: on average over the three crop year, treatments with sorghum (Sorghum and Sorghum + Cowpea) combined with 0 kg N ha⁻¹ resulted in a 9% reduction in grain protein content compared to the bare soil control at 0 kg N ha⁻¹ (Figure 2). Similarly, with 150 kg N ha⁻¹ fertilization, sorghum treatments showed a 5% reduction in grain protein content compared to bare soil control at the same N rate.

Furthermore, wheat grain protein content after Sorghum shows a reduction of 1.1% compared to bare soil control in 2021; this difference reaches a reduction of 10.9% in 2022, decreasing in 2023 with -8%. Conversely, the effect of N levels on grain protein content is much less volatile in crop year, showing differences ranging from -12 to -13% of the control with 0 kg N ha⁻¹ compared to 150 kg N ha⁻¹.

3.6 Nitrogen use efficiency

Ground cover treatments involving biomass production during the intercrop period (natural grassing, Sorghum, Sorghum + Cowpea, and Niger + Cowpea) led to a significant reduction in

TABLE 4 Effect of ground cover (GC) management during intercropping periods and nitrogen fertilization rate (N rate) on wheat grain yield, test weight (TW) and grain protein content (GPC) in 3 crop years (CY).

Factor	Source of variation	Grain yield (t DM ha ⁻¹)	TW (kg hl ⁻¹)	GPC (%)
GC	T	6.54 a	79.04 b	14.31 a
	NT	5.30 c	79.01 b	14.03 a
	S	5.03 c	79.94 a	13.16 b
	SV	5.00 c	80.16 a	12.94 b
	NV	5.68 b	79.92 a	13.18 b
	p-value	<0.001	<0.001	<0.001
N rate	0	4.32 c	79.89 a	12.47 c
	90	5.37 b	79.54 a	13.47 b
	150	6.41 a	79.51 a	14.29 a
	p-value	<0.001	0.297	<0.001
CY	2021	7.91 a	81.55 a	12.33 c
	2022	5.59 b	78.19 b	13.13 b
	2023	4.15 c	79.97 c	14.52 a
	p-value	<0.001	<0.001	<0.001
GC x N	p-value	0.031	0.07	0.023
GC x CY	p-value	<0.001	0.004	0.005
N x CY	p-value	<0.001	0.023	0.043
GC x N x CY	p-value	0.004	0.062	0.176
SEM		0.14	0.13	0.10

For each factor, values followed by different letters are significantly different. The level of significance (*p*-value) is reported in the table.

Ground Cover: T, bare soil control; NT, natural grassing; S, Sorghum; SV, Sorghum + Cowpea; NV, Niger + Cowpea.

N rate: total nitrogen rate applied to wheat as top dressed ammonium nitrate between tillering and the stem elongation growth stages (0, 90, 150 kg N ha⁻¹).

Field experiment carried out in the 3 Crop Years (CY).

SEM, standard error of means.

PPF and PNB, with a 12% and 18% decrease, respectively, compared to the bare soil control (Table 5). The most pronounced decline in PNB was observed in sorghum-based treatments (Sorghum and Sorghum + Cowpea), while natural grassing and Niger + Cowpea treatments, although 14% lower than bare soil control, did not differ significantly from each other. For both PPF and PNB, fertilization at 150 kg N ha⁻¹ resulted in lower efficiency values compared to 90 kg N ha⁻¹. Both indices also declined progressively across crop years. Significant interactions were detected between ground cover × crop year, N rate × crop year, and ground cover × N × crop year (Supplementary Table 8). Both AE and RE were significantly affected by ground cover management (Supplementary Table 9). Wheat following Sorghum showed the highest AE (20.13) and RE (58.70), significantly greater than the bare soil control, corresponding to increases of approximately 183% for AE and 98% for RE relative to bare soil control. Intermediate values were observed for natural grassing, Sorghum + Cowpea, and Niger + Cowpea treatments. In addition, both indices declined over the three crop years, with AE decreasing from 18.56 in 2021 to 9.52 in 2023 (−49%) and RE decreasing from 64.33 to 32.49 (−49%), indicating a strong year effect on nitrogen use efficiency.

3.7 Element concentration in wheat grain

3.7.1 Macro, meso and micronutrients

The concentrations of P and K in grain after CC (Sorghum, Sorghum + Cowpea, and Niger + Cowpea) showed no significant differences with bare soil control (Supplementary Table 10). Natural grassing and Niger + Cowpea had a P content 5% higher in comparison with bare soil control, Sorghum and Sorghum + Cowpea, while K content averaged 3 g kg⁻¹ across treatments, and no significant effects were reported from ground cover. No significant differences were observed for the N rate factor on macronutrient content.

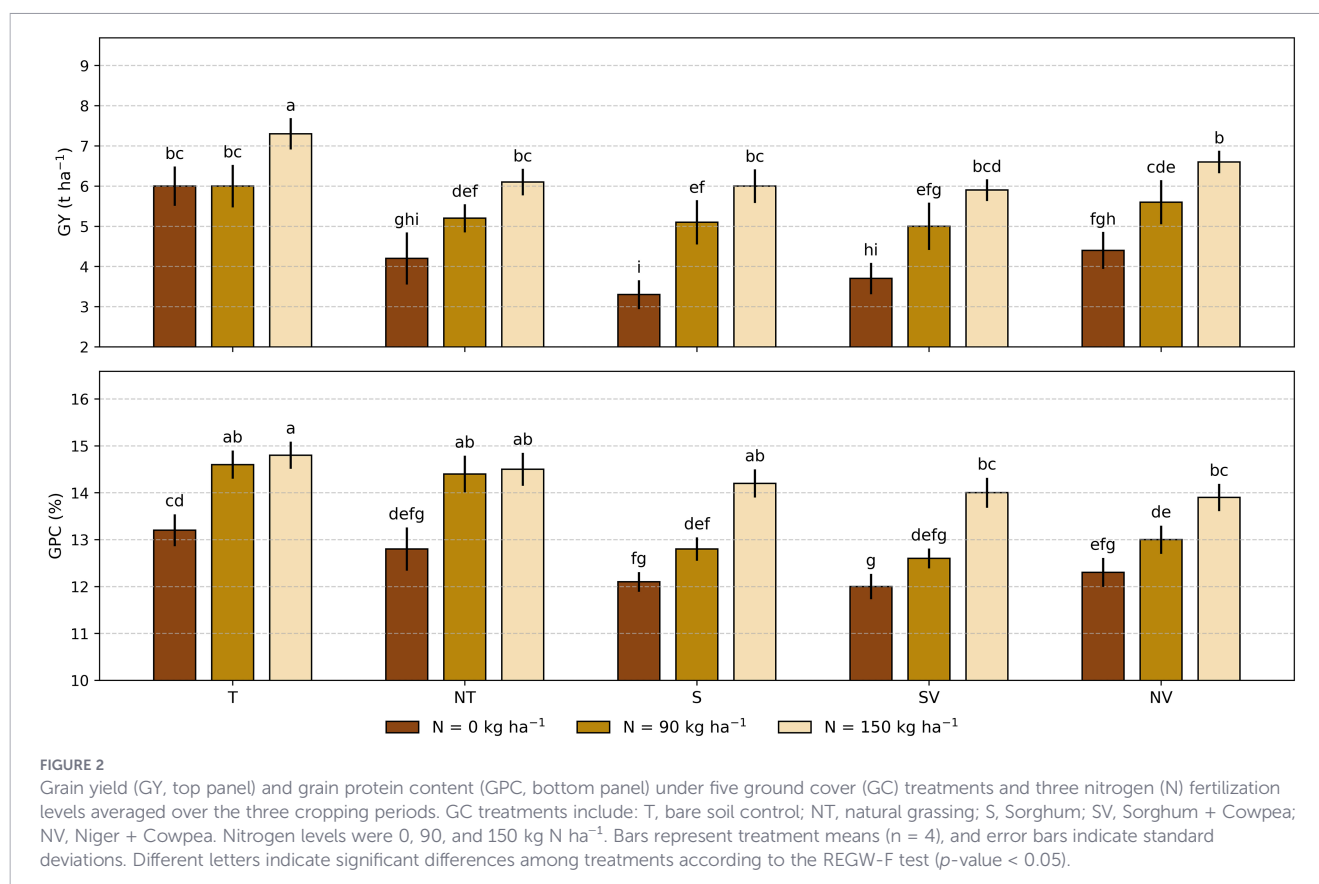
Calcium and Mg concentrations in the grain averaged 350 mg kg⁻¹ and 900 mg kg⁻¹, respectively (Figure 3). ground cover treatments significantly affected Ca levels: CC treatments (Sorghum, Sorghum + Cowpea and Niger + Cowpea) reported no significant differences between them and reduced the Ca content in the grain compared to bare soil control (−16%), while natural grassing is shown to be 8% lower than the bare soil control. In contrast, Mg content increased significantly with the biomass-producing treatments, reporting an increase of 11.5% compared with bare soil control. The Ca: Mg ratio also decreased significantly by 23% in CC treatments compared with bare soil control. The N rate further influenced grain Ca and Mg levels, with higher fertilization rates leading to increased concentrations. The reduction in Ca concentration, combined with the increase in Mg, resulted in a marked overall shift in the Ca: Mg balance of the grain driven by ground cover treatments.

Ground cover treatments involving biomass production (natural grassing, Sorghum, Sorghum + Cowpea, and Niger + Cowpea) significantly increased Mn and Cu levels in wheat grain (Table 6), with Mn increasing by about 10% and Cu by about 20%, compared with the bare soil control. Fe content varied among treatments, with Sorghum and Niger + Cowpea showing the lowest concentrations. The wheat N fertilization rate significantly and proportionally increased Cu and Zn levels in the grain, by +4% and +20%, respectively, compared to the unfertilized control.

3.7.2 Potentially toxic elements

Grain concentrations of PTEs were assessed, in the third growing season, revealing treatment-specific differences (Supplementary Table 11). For Ni, the Sorghum + Cowpea treatment showed significantly lower concentrations than the control (−39%), while the other ground cover treatments showed intermediate levels. In contrast, Cd concentrations were significantly higher in the natural grassing treatment than in the bare soil control (+22%), while the other treatments, including Niger + Cowpea, showed values close to the control.

Fertilization with N resulted in a significant increase in Ni and Cd content in grain: no significant differences are shown between 90 and 150 kg N ha⁻¹ for Ni, but grain reports concentrations lower than 25% compared to the unfertilized control; conversely, for Cd, the treatment with 90 kg N ha⁻¹ reports a significantly higher



value than the treatment at 150 kg N ha⁻¹ and the control with 0 kg N ha⁻¹, +16% and +39%, respectively.

4 Discussion

Summer CC introduction in a cereal system based on a wheat monoculture presented important effects related to CC biomass characteristics and soil fertility changes, which impacted on wheat yield and quality.

4.1 Cover crop biomass

Biomass production during the three intercrop periods underlined the strong dependence of summer CC on meteorological conditions (Błażewicz-Woźniak and Wach, 2012). The significantly higher biomass production recorded in the first intercrop period was favored by a summer with milder temperatures and higher soil water availability at sowing time.

Across the three intercrop periods, sorghum-based treatments (Sorghum and Sorghum + Cowpea) showed a significant capacity to produce biomass, which translated into the largest inputs of C to soil. However, sorghum biomass exhibited a significantly high C/N ratio, markedly higher than the legume-rich Niger + Cowpea treatment, slowing down plant residue mineralization (Lynch et al., 2016). The possible use of Sorghum + Cowpea mixture to improve the degradability of the CC biomass was ineffective, due to an imbalance in establishment speed and early vigor between the

species, which had always enhanced a higher development of sorghum compared to cowpea. Treatment labeled as Niger + Cowpea reported a lower biomass-producing capacity than the treatments that included the use of sorghum (Sorghum and Sorghum + Cowpea), but this ground cover did not show significant differences with the natural grassing treatment, both in biomass produced and C/N ratio.

Despite lower tissue concentrations of N and P, the sorghum residues returned a similar N and P amount to soil in comparison to other ground cover due to the high biomass productivity. Potassium uptake, on the other hand, was actually highest in the Niger + Cowpea mixture, likely because of the asteraceae scavenging capacity. The treatment left to natural grassing by spontaneous flora did not show significant differences with the Niger + Cowpea treatment in terms of biomass and nutrient uptake performance, but this practice is certainly unfavorable in terms of seed bank enrichment and weed specialization, raising long-term management concerns.

4.2 Effect on soil organic matter

The introduction of CC significantly influenced SOM dynamics in a 3-year period, as evidenced by the observed increase in soil C stocks in ground cover treatments compared to the bare soil control. This improvement may be attributed to the consistent input of organic material from CC residues, which likely contributed to the accumulation of C in soil (Büchi et al., 2018). More than 70% of C was stored in the MAOM pool, which is commonly associated with longer-term stabilization relative to

TABLE 5 Effect of ground cover (GC) management during intercropping periods and nitrogen fertilization rate (N rate) on partial-factor productivity (PFP) and partial nitrogen balance (PNB) in 3 crop years (CY).

Factor	Source of variation	PFP	PNB
GC	T	58.00 a	1.42 a
	NT	50.20 c	1.22 b
	S	49.52 c	1.12 c
	SV	50.46 c	1.08 c
	NV	54.04 b	1.23 b
	<i>p</i> -value	<0.001	<0.001
N rate	0	- -	- -
	90	63.39 a	1.38 a
	150	42.83 b	1.04 b
	<i>p</i> -value	<0.001	<0.001
CY	2021	73.78 a	1.65 a
	2022	53.00 b	1.25 b
	2023	37.62 c	0.95 c
	<i>p</i> -value	<0.001	<0.001
GC x N	<i>p</i> -value	0.068	0.383
GC x CY	<i>p</i> -value	<0.001	<0.001
N x CY	<i>p</i> -value	<0.001	<0.001
GC x N x CY	<i>p</i> -value	<0.001	<0.001
SEM		1.7	0.03

For each factor, values followed by different letters are significantly different. The level of significance (*p*-value) is reported in the table.

Ground Cover: T, bare soil control; NT, natural grassing; S, Sorghum; SV, Sorghum + Cowpea; NV, Niger + Cowpea.

N rate: total nitrogen rate applied to wheat as top dressed ammonium nitrate between tillering and the stem elongation growth stages (0, 90, 150 kg N ha⁻¹).

Field experiment carried out in the 3 Crop Years (CY).

SEM, standard error of means.

particulate pools (Kauer et al., 2021). Microbial activity, stimulated by the steady supply of C and nutrients from CC, might have enhanced the stabilization of organic inputs (Bucka et al., 2021). The decomposition of residues and microbial turnover could have indeed produced low-molecular-weight organic compounds and metabolites that readily bind to mineral surfaces, increasing the MAOM fraction (Lavallee et al., 2020). Additionally, sorghum-based treatments with high C/N ratios may also have contributed to greater accumulation in the fPOM fraction due to slower decomposition and increasing selection of recalcitrant organic residues (Ntonta et al., 2024).

CC introduction also increased soil N stocks within a 3-year period, though the effect varied depending on biomass characteristics. Treatments with low C/N biomass (Niger + Cowpea and natural grassing) led to greater N accumulation compared to high-C/N biomass treatments (Sorghum and Sorghum + Cowpea), likely due to more efficient decomposition and microbial processing. In contrast, high-C/N residues may have caused temporary N immobilization, limiting their contribution to soil N gains (Bilenky et al., 2022). The influence of fertilization rate on N retention revealed a shift in allocation, with higher N rates promoting the nutrient accumulation in the more labile fPOM and oPOM fractions. However, the lack of significant changes in the

MAOM fraction under varying N rates suggests that the long-term benefits of ground cover integration may be realized independently of fertilization practices (Fohrafellner et al., 2024).

4.3 Agronomic and qualitative effects on wheat

Across the three crop years, biomass incorporation and its effect on soil clearly affected wheat growth, as shown by lower value of AUCGC and plant height and a significant impact on grain yield and yield components (spike density, kernels per spike and TKW). Interestingly, straw biomass did not differ among CC treatments and HI actually increased compared to the bare soil control, indicating that during this initial period, wheat partially compensated for smaller canopies by allocating a greater share of resources to grain. These results suggest that residue-driven N immobilization during wheat growth is linked to an early deficiency which translates into smaller vegetative structures and, ultimately, lower grain yield whenever CC biomass is incorporated without an adequately synchronized N supply. It is also evident that high-C/N biomass (Sorghum and Sorghum + Cowpea) had a greater effect on wheat development, clearly affecting AUCGC during the whole wheat growth and overall biomass production (Miyamoto et al., 2020). Data collected in the 3-year period highlighted that CC introduction could affect N availability for wheat during all the crop growth, since it resulted in a significant impact on both the tillering stage (low ear density, according to Scapino et al., 2025) and the stem elongation stage (low number of grains per ear and reduced plant height). In accordance with the yield components related to the ripening stage (TKW), it seems that the effect of ground cover treatment led to a lower impact on N deficiency during the late wheat growth stages. Furthermore, the slight increase of TKW and TW observed as a consequence of CC introduction is due to a compensatory mechanism in the wheat plant itself, which compensates for the higher production of grains per square meter with lighter kernels (Tillett et al., 2022; Sadras, 2007; Xie and Sparkes, 2021), and which had masked the possible real impact of reduced N availability also in the final wheat cycle. The strong effect of CC on grain protein content, which depends on remobilization of N absorbed during vegetative stages and is also strongly associated with late N uptake during ripening (Yu et al., 2018), suggests that N immobilization caused by incorporation of CC biomass persists into wheat ripening.

The NUE indices directly relating GY to applied N (PFP and PNB) indicate that the ground cover factor reduced fertilizer use efficiency whenever biomass was produced during the intercrop period, with the strongest penalties observed in grass-dominated options (Sorghum, *Sorghum × drummondii*). In contrast, indices integrating GY, applied N, and its availability to the crop as influenced by the ground cover × N-rate legacy effect (AE and RE) showed that wheat grown after S, particularly after three consecutive seasons of CC biomass production, experienced more pronounced N deficiency, especially under unfertilized conditions (N0). This resulted in a greater apparent efficiency of applied N under fertilized treatments. This pattern is consistent with residue-driven N immobilization from high C/N inputs, which depresses short-term

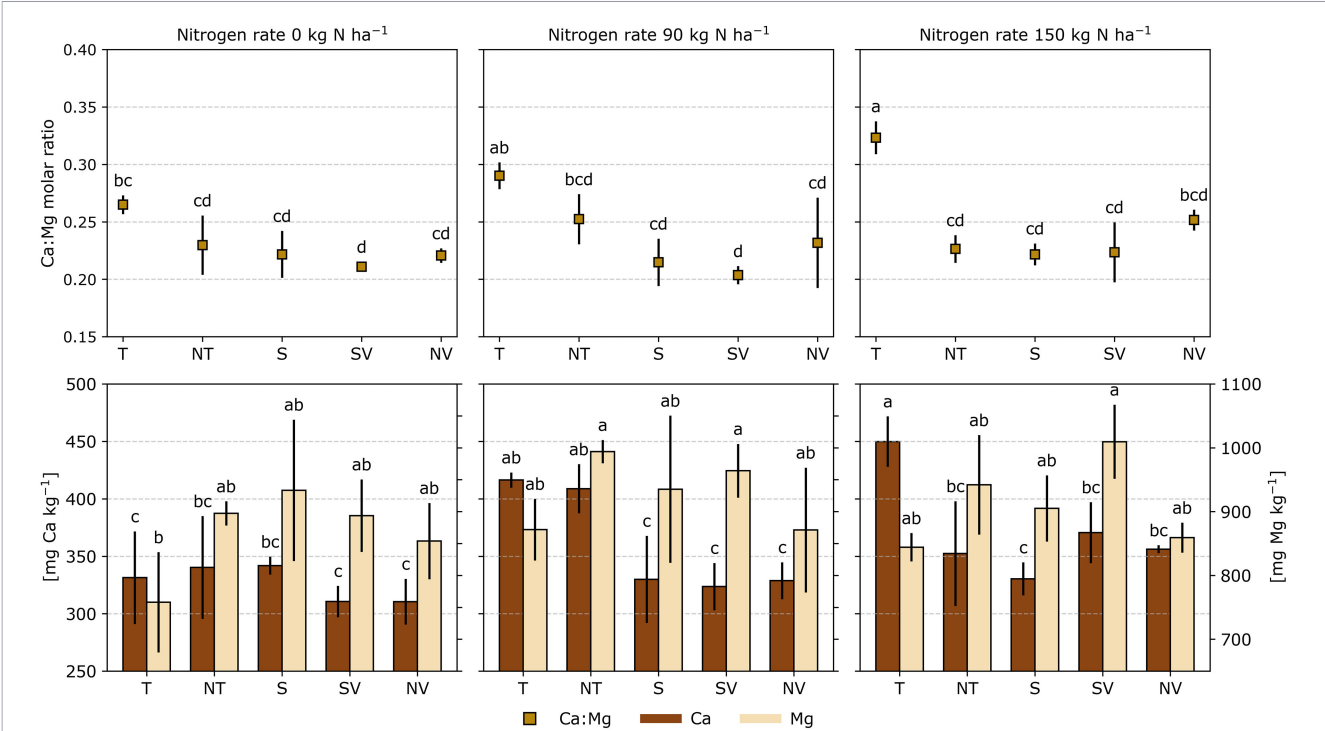


FIGURE 3
Calcium-to-magnesium (Ca: Mg) molar ratio and grain nutrient concentrations under five ground cover (GC) treatments implemented during the third cropping periods (2022–2023) across three nitrogen fertilization levels (0, 90, 150 kg N ha⁻¹). (Top row) Ca: Mg molar ratio in wheat grain. (Bottom row) Grain calcium and magnesium grain concentrations. The left y-axis shows Ca concentration; the right y-axis shows Mg concentration. GC treatments include: T, bare soil control; NT, natural grassing; S, Sorghum; SV, Sorghum + Cowpea; NV, Niger + Cowpea. Bars represent means (n = 4), and error bars indicate standard deviations. Within each parameter, different letters indicate significant differences among treatments according to the REGW-F test (p-value < 0.05).

N availability to wheat and helps explain the concomitant declines in yield and grain protein content (Miyamoto et al., 2020). The only treatment that did not involve the use of grass (Niger + Cowpea) attenuated these penalties on yield but did not prevent the decline in grain protein, indicating that N limitation persisted into late growth stages despite the lower residue C/N at termination. Thus, residue quality modulates but does not remove the temporal mismatch between N release and crop demand. N application moderated, but did not eliminate, the negative effects on grain yield. Grass-based CC still underperformed relative to bare-soil control, and gains came at

TABLE 6 Effect of ground cover (GC) management during intercropping periods and nitrogen fertilization rate (N rate) on wheat grain micronutrient content (Fe, Mn, Cu, Zn) in the third crop year (2023).

Factor	Source of variation	Fe mg kg ⁻¹	Mn mg kg ⁻¹	Cu mg kg ⁻¹	Zn mg kg ⁻¹
GC	T	28.24 ab	23.28 c	3.32 c	15.70 a
	NT	28.42 a	27.04 a	3.84 ab	19.05 a
	S	24.75 ab	25.63 abc	4.08 a	19.53 a
	SV	28.25 a	26.14 ab	4.11 a	19.69 a
	NV	24.10 b	23.30 bc	3.74 b	17.22 a
	p-value	0.04	0.009	<0.001	0.069
N rate	0	25.82 a	25.10 a	3.70 b	16.10 b
	90	28.28 a	25.28 a	3.80 a	18.90 a
	150	26.31 a	24.85 a	3.90 a	19.70 a
	p-value	0.211	0.894	0.012	0.015
GC x N	p-value	0.682	0.014	0.121	0.724
SEM		0.61	0.48	0.06	0.55

For each factor, values followed by different letters are significantly different. The level of significance (p-value) is reported in the table. Ground Cover: T, bare soil control; NT, natural grassing; S, Sorghum; SV, Sorghum + Cowpea; NV, Niger + Cowpea. N rate: total nitrogen rate applied to wheat as top dressed ammonium nitrate between tillering and the stem elongation growth stages (0, 90, 150 kg N ha⁻¹). Field experiment carried out on the wheat grain of the third crop year (2023). SEM, standard error of means.

the expense of lower PFP/PNB. These findings indicate that reliance on elevated nitrogen (N) inputs is suboptimal. Instead, management should prioritize strategies to enhance synchronization between mineralization and crop uptake.

The study has highlighted that, in addition to the effects on yield, the inclusion of CC in a cereal cropping system has determined even more important effects on the quality traits, with further important implications for the economic convenience of cash crop. Specifically, the grain protein content remained above the qualitative threshold indicated by the food chain to confer the product as high protein wheat (grain protein content >14%) (Landolfi et al., 2021) only for the bare soil control and natural grassing treatment, while the treatments involving CC sowing (Sorghum, Sorghum + Cowpea, and Niger + Cowpea) resulted in a reduction of protein more than 1%, and comparable to that determined by the N fertilization of 90 kg N ha⁻¹. The interaction between ground cover and N rate factor suggests that in the first three years of CC introduction, fertilization management must take into account a reduction in N efficiency, especially at low fertilization rates (90 kg N ha⁻¹).

Additionally, the chemical composition of wheat grain was influenced by the interaction of ground cover and N rate treatments, revealing distinct impacts on macro-, meso-, and micronutrient concentrations. CC may have limited influence on P cycling in the short term due to its slower cycling rates compared to N (Hansen et al., 2021). However, the slight differences in P content, particularly the higher levels observed in the natural grassing compared to the Niger + Cowpea treatment, may be explained by variations in root system dynamics and P allocation (Maltais-Landry et al., 2014). The observed uniformity in K levels across treatments likely reflects high baseline soil K levels, which were sufficient to meet wheat nutrient requirements, minimizing the impact of CC on grain K content. The response of mesonutrients Ca and Mg to ground cover treatments can be partly explained by cation interactions and nutrient release patterns. In the studied soil, characterized by neutral pH, moderate CEC, a marked dominance of exchangeable Ca (58%) over Mg (4.2%), and the initial Ca/Mg ratio (14.0) indicates a Ca-dominated exchange complex. Under these conditions, the increase in grain Mg observed under CC implementation likely reflects enhanced mobilization and uptake of Mg rather than Ca depletion. Both Ca²⁺ and Mg²⁺ ions compete for uptake at the root surface due to similar charge and ionic radius (Mengel et al., 2001). Cover crops with deep rooting systems may enhance Mg availability by accessing the element from deeper soil horizons, potentially increasing Mg uptake in subsequent wheat crops (Miguez and Bollero, 2005). Additionally, the decomposition of CC residues can release Mg through mineralization processes. In contrast, Ca tends to bind more strongly to organic matter and soil colloids (Nair, 2002), which may reduce its immediate bioavailability relative to Mg under certain soil conditions. Given the low organic matter content and rapid mineralization rate, improved Mg acquisition may signal greater biological activation of nutrient pools through residue decomposition and rhizosphere processes (Hansen et al., 2021; Scavo et al., 2022). These combined factors can shift the Ca: Mg uptake balance toward Mg. From an agronomic and nutritional perspective, increased Mg concentration may represent a positive

outcome, suggesting more efficient cation cycling within the soil-plant system (Büchi et al., 2018). Micronutrient concentrations showed a pronounced response to CC treatments, with increases in Mn, Cu, and Zn levels, suggesting enhanced micronutrient bioavailability under ground cover treatments. This increase could be attributed to the impact of decomposing CC residues on rhizosphere pH and chelation processes, thereby facilitating micronutrient solubility and uptake (Grüter et al., 2019). Regarding PTEs, the lower Ni concentrations observed in the Sorghum + Cowpea treatment and the steady Cd levels in the CC treatments may both be attributed to higher organic matter inputs, which promote metal immobilization and reduce plant uptake (Grüter et al., 2019). Overall, these findings indicate that the nutritional benefit of CC in the first three years of introduction in a monosuccessional approach of wheat is limited to an increase in micronutrient content, while grain yield and grain protein content show a significant decline and no significant rise in PTE uptake.

4.4 Agronomic considerations in the first three years of summer CC

Building on the positive effects observed on soil functionality and SOM accumulation, notwithstanding the initial decline in wheat profitability during the first three years following the introduction of CC, we expect that, over time, the “cash crop - CC - soil” system will reach a state of stabilization. At this stage, the performance gap between biomass-producing treatments and the control is expected to narrow, enabling the benefits of SOM build-up and legume-derived N inputs to be fully expressed in terms of wheat yield and quality. However, several considerations are crucial in order to reduce the incidence of negative effects of CC in the first years of introduction: a) providing for a different fractionation and an early advance of the first N inputs to the cash crop could help mitigate N sequestration resulting from the degradation of CC biomass; b) the selection of appropriate CC species or mixtures is crucial for ensuring the profitability of the subsequent cash crop (e.g., the introduction of legumes). Equally important, however, is the early vigor of the chosen species, since inadequate growth and low competitiveness against weeds may compromise the entire investment made up to the point of CC sowing; c) the timing of CC termination should be based on the phenological stage of the main species, considering biomass degradability in terms of quality rather than quantity, as is well established for forage crops (Li et al., 2022), in order to reduce the C/N ratio of plant tissues incorporated into the soil; d) under climate change scenarios, the establishment of CCs during summer is expected to be increasingly delayed, making it crucial to minimize the interval between wheat harvest and CC sowing, and to adopt appropriate equipment and techniques that can reduce costs while preserving soil water.

5 Conclusions

This study provides robust, field-scale evidence that integrating summer CC into continuous wheat systems can deliver rapid,

durable C gains while reshaping N dynamics and soil fertility profiles. In just three years, almost 6 Mg C ha⁻¹ was sequestered, most of it in the MAOM pool, indicating real potential for long-term storage (hypothesis (i)). However, not all CC led to the same effects over this period. High-biomass and high-C/N species, such as sorghum, generated strong C returns but imposed steep agronomic penalties, delaying N availability, resulting in lower wheat vigor, reduced yield, and lower grain quality (hypothesis (ii)). In contrast, a non-grass-based mixture (Niger + Cowpea) struck a better balance, maintaining moderate C inputs with less disruption to yield and soil N cycling, but reducing the amount of OM incorporated into the soil.

Crucially, this work quantifies the N trade-offs that define the practical boundary between C sequestration and productive cropping. While modest N inputs (90 kg N ha⁻¹) restored yield and preserved NUE in non-grass-based rotations, higher rates needed to support sorghum reversed gains in SOM stability, redirecting C toward more labile pools. These findings underscore a clear trade-off: pushing productivity and qualitative risks undercutting climate benefits unless the whole cropping system is adequately managed (hypothesis (iii)). Thus, it is expected that legumes or legume-heavy CC, early termination of high-C/N species, and targeted N management could emerge as key levers. Beyond C, CC improved total soil N and micronutrient density in grain, indicating broader fertility benefits. Future studies will need to be conducted to describe the dynamics of N availability for cash crops in succession and to develop models capable of guiding the nutrition of wheat step by step based on the main parameters that characterize the biomass of CC.

To our knowledge, this is the first multi-year South Europe study, to integrate species and N-rate interactions with SOM fractionation and a complete wheat performance metrics. It provides essential coefficients and insights for scaling carbon-smart rotations into climate policy frameworks, particularly those driving the EU 2050 neutrality goal. Future research should extend monitoring to track MAOM stabilization and alternative CC and N strategies, to enhance system efficiency without escalating inputs or reducing wheat productivity and quality.

Data availability statement

The original contributions presented in the study are included in the article/[Supplementary Material](#). Further inquiries can be directed to the corresponding author.

Author contributions

PC: Investigation, Writing – original draft, Formal analysis, Data curation. RC: Visualization, Investigation, Formal analysis, Data curation, Writing – original draft. SM: Formal analysis, Investigation, Writing – review & editing. RM: Investigation, Writing – review & editing, Formal analysis. AR: Funding acquisition, Project administration, Writing – review & editing,

Supervision. MM: Resources, Funding acquisition, Writing – review & editing, Methodology. LC: Resources, Funding acquisition, Methodology, Validation, Writing – review & editing. MB: Conceptualization, Supervision, Data curation, Investigation, Writing – review & editing, Methodology, Funding acquisition, Validation, Formal analysis.

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Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

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Glossary

AE	Agronomic Efficiency	N	nitrogen
ANOVA	Analysis of Variance	NaPT	sodium polytungstate
AUCGC	Area Under Canopy Greenness Curve	NDVI	Normalized Difference Vegetation Index
C/N	carbon-to-nitrogen ratio	NT	ground cover left to natural grassing by weeds during intercropping period
C	carbon	NUE	Nitrogen Use Efficiency
CC	Cover Crop	NV	ground cover with cowpea and niger mix (50% plant/plant) in intercropping period
CEC	cation exchange capacity	oPOM	occluded Particulate Organic Matter
CY	Crop Year	P	phosphorus
DM	dry matter	PFP	Partial-factor Productivity
ET ₀	Potential Evapotranspiration	PNB	Partial Nitrogen Balance
fPOM	free Particulate Organic Matter	PTEs	Potentially Toxic Elements
GC	Ground Cover	RE	Recovery Efficiency
GDD	Growing Degree Days	REGW-F	Ryan-Einot-Gabriel-Welsh F
GPC	Grain Protein Content	S	ground cover with pure sorghum (100%) in the intercropping period
GS	growing stage	SEM	Standard Error of the Mean
GY	Grain Yield	SOM	soil organic matter
GY ₀	grain yield of unfertilized wheat (0 kg N ha ⁻¹)	SV	ground cover with sorghum and cowpea mix (50% plant/plant) in intercropping period
GY _f	grain yield of fertilized wheat (90 or 150 kg N ha ⁻¹)	T	ground cover with glyphosate application in intercropping period
HI	Harvest Index	TKW	Thousand Kernel Weight
IP	Intercrop Period	TW	Test Weight.
K	potassium		
MAOM	Mineral-Associated Organic Matter		