

Article

Exploring Winter Legume Cover Crop Management Strategies in Irrigated Maize Monoculture Systems

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Abstract

Management of legume cover crops to reduce their cost by using no-tillage and reducing seed rate could increase their adoption. Despite the growing interest in cover crops, no information exists simultaneously regarding the potential of different species and how the sowing method and seed rate affect nitrogen (N) contribution and the yield of the subsequent maize crop. During a four-year field trial, under irrigated conditions in the Ebro valley (NE Spain), three leguminous cover crop species (pea, common vetch and hairy vetch), two cover crop seeding methods (conventional tillage and no-tillage) and two seeding rates (normal and 25% reduced) were tested and compared with a control treatment without a cover crop. The aboveground cover crop biomass and the N derived from biological fixation (BNF); aboveground biomass and total N in weeds; soil mineral nitrogen; and the effect on maize grain yield and N content were evaluated. Pea and common vetch produced more biomass (+76%) and had a higher N uptake (+50 to 60%) compared to hairy vetch. The sowing of the cover crops after no-tillage combined with a reduced sowing rate reduced biomass production by 14%. The percentage of nitrogen derived from the atmosphere (Ndfa) was above 60% for all species and the differences in total N derived from biological fixation (BNF) among treatments were related to the aboveground biomass. The introduction of cover crops reduced weed growth compared to the control especially in the no-tillage treatment. Cover crops increased maize grain yield by 12% and N uptake by 17% compared to the control treatment without a cover crop.

Keywords: no-tillage; sowing rate; pea; vetch; nitrogen fixation



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1. Introduction

The establishment of winter leguminous cover crops under irrigated conditions, replacing the traditional winter fallow–maize system, may be a win-win strategy to support the objectives of the Farm-to-Fork strategy, reducing N fertilizer use and nutrient losses in agriculture.

Incorporating the biomass of leguminous cover crops into the soil as green manure enables a reduction in N fertilizer dependency through biological nitrogen fixation, thereby enhancing nitrogen use efficiency (NUE) and mitigating the environmental impact associated with N production and application [1–4]. In addition, the cover crops can contribute to the reduction in soil erosion, improving soil structure and water retention, and to crop diversification of simplified cropping systems [5–7]. Despite being included as a recommended practice in the most recent Common Agricultural Policies of the European Union

(2014–2020 and 2021–2027), it is difficult to calculate the actual adoption of cover crops (CCs) at the European level [8]. According to [9], using modelling that combines satellite data with statistical surveys, the use of CCs tends to be higher in the regions corresponding to Northern France, Germany and Denmark, but the global use in Europe is still very low, with CCs below 5% acreage in most countries. The costs of adopting CCs due to extra labour, machinery, and seeds can prevent their use [10]. In addition, the implementation of cover crops can present certain disadvantages for farmers, such as a lack of knowledge on how to manage them [8]. Furthermore, the labour required for introducing CCs, especially after a maize crop, is constrained by the narrow temporal window after harvest [11]. Other authors [7] highlighted the need for information regarding the beneficial effects of introducing CCs into the rotation.

In many irrigated areas of Spain, maize yields are around 15 Mg ha⁻¹ of grain [12–14], with N fertilizer rates ranging from 250 to 450 kg N ha⁻¹ year⁻¹ [12,15]. However, this rate can be significantly reduced without yield penalties [16–19] depending on soil conditions, irrigation system efficiency and legume precedents. In these intensively irrigated systems, the introduction of leguminous cover crops allows farmers to reduce fertilizer use by at least 50 kg N ha⁻¹ year⁻¹, improving the nitrogen use efficiency (NUE) of the system [1,4,20]. In these monoculture maize systems, long-season maize cultivars (CRM > 115) are grown from April to October, leaving a short period for leguminous CC sowing due to the generally wet soil conditions, rain events, and decreasing air temperature during the autumn. The high amount of maize crop residues increases farmers' perception of the difficulty of cover crop sowing with no-tillage, so it is not a common practice. However, the use of conventional tillage prior to cover crop seeding can present some environmental and management disadvantages compared to the traditional winter fallow. From an environmental perspective, conventional tillage for sowing increases soil disturbance [21] and greenhouse gas emissions, primarily from fuel use [4]. From a management perspective, conventional tillage for sowing the CCs requires additional machinery use and a delay in the sowing date compared to no-tillage. Hence, sowing CCs with no-tillage seems to be an interesting strategy to introduce CCs, with potential environmental, economic, and agronomic benefits. However, the advantages of directly sowing CCs compared to conventional sowing must be evaluated for the specific soil and weather conditions and consider the inherent characteristics of different cover crop species. Previous studies [20] under similar conditions indicate that no-tillage sowing allowed for earlier planting dates, but the effect on CC biomass was not consistent among years due to greater problems with CC establishment with no-tillage.

The choice of cover crop species to maximize biomass production depends mainly on the edaphoclimatic conditions and the length of the growing season between the harvest and planting of the main crop [7,22]. Vetch and winter peas are leguminous species that are well adapted to Mediterranean environments, frost-tolerant, and already cultivated in rain-fed and irrigated areas of Spain as main crops for grain or forage production. Among them, winter peas are more adapted than vetch to late sowing in autumn. Thus, these species are strong candidates for inclusion as cover crops in the monoculture maize systems of Spain. The production of aboveground biomass by cover crops is positively related to many of the benefits of cover crops, such as high soil coverage, weed suppression [23,24], and N accumulation [25,26]. To prevent poor CC establishment due to poor soil conditions or excessive amounts of crop residues, the CC sowing seed rate is usually increased, increasing the associated costs of the cover crop, hindering its adoption. Thus, assessing the feasibility of reducing the standard seeding rates of the CC is relevant for seed cost reduction.

It is well established that symbiotic nitrogen fixation is energetically more costly than soil inorganic nitrogen uptake by legume plants [27] and that low soil inorganic

N availability promotes a higher percentage of nitrogen derived from the atmosphere (Ndfa, %) in legumes [28,29]. Most research studies report the potential of direct sowing to enhance nitrogen fixation due to the reduced nitrogen availability, improved water retention and improved soil physical structure [30]. Furthermore, no-tillage generally increases nodulation and total nitrogen fixation compared to conventional tillage [30–32]. However, as a review [30] points out, no-tillage can also negatively affect nitrogen fixation depending on soil and management conditions and must be assessed in different agroecosystems.

To date, no study of CCs conducted under Mediterranean irrigated conditions has concurrently evaluated the three factors addressed in this work—sowing method, seeding rate, and cover crop species. The study provides empirical data that will enable farmers and future research to optimize the management of leguminous cover crops promoted by European agronomic policies. Therefore, the objective of this study was to assess, in a monoculture maize system, how the choice of the legume cover crop species, the sowing rate (standard and reduced), and the sowing type (conventional vs. no-tillage) impact the cover crop's development and its nitrogen fixation and their effect on the grain yield of the subsequent maize crop under the semi-arid irrigated conditions of the Ebro Valley (NE Spain).

2. Materials and Methods

2.1. Experimental Site

The experiment was performed over four years (2019–2022) in a 2 ha field equipped with a solid-set sprinkler irrigation system at the Agrifood Research and Technology Centre of Aragón (CITA), located in Zaragoza (Ebro Valley, NE of Spain; 41°39' N 0°53' W; 221 m altitude). The area has a Mediterranean continental climate with a 14.1 °C average temperature, 298 mm total annual precipitation and grass reference crop evapotranspiration (ET₀) of 1243 mm. The soil is a Typic Xerofluvent fine-textured soil (soil survey staff, 2014). The properties of the soil are described in detail in [33]. Briefly, the soil is deep (1.2 m), calcareous (30–40% CaCO₃) sandy loam, and high-pH (8.1–8.4), with low soil organic matter (1.9% at 0–30 cm) and with 51, 36, and 13% sand, silt, and clay, respectively, in the upper part (0–30 cm) of the soil profile. The levels of phosphorous (P Olsen = 29 mg P kg⁻¹) and extractable potassium (164 mg K kg⁻¹) in the topsoil (0–30 cm) are within the range of sufficiency for maize crop. The monthly temperature averages and cumulative precipitation during the experiment are presented in Figure 1.

2.2. Treatments, Experimental Design and Crop Management

The experiment assessed the effect of winter legume cover crops on a monoculture maize system under sprinkler-irrigated conditions. The factors analyzed in the study were the species of the cover crop, the rate of seeding of the cover crop, and the sowing method of the cover crop. The three cover crop species evaluated were peas (P) (*Pisum sativum* var. 'Enduro'), common vetch (CV) (*Vicia sativa* var. 'Serva') and hairy vetch (HV) (*Vicia villosa* var. 'Goliat'). An additional treatment without a cover crop was included as a control (C). In this treatment, weeds were allowed to grow during the winter fallow period. The cover crop sowing densities were denominated as 'normal' (N) and 'reduced' by 25% (R). The considered normal densities were 200 kg ha⁻¹ for peas, 100 kg ha⁻¹ for common vetch, and 50 kg ha⁻¹ for hairy vetch, according to the usual sowing rates used in the region for these crops as a sole crop. The target seeding rate for each cover crop species was adjusted on the commercial drill model SD-1203 (Sola S.L., Calaf, Barcelona, Spain). After the previous maize harvest, maize residues were chopped. The cover crop sowing methods were as follows: (1) sowing after conventional tillage (CT), using a disc harrow or cultivator,

depending on soil conditions, and (2) sowing with no-tillage (NT). Prior to the present study, the management of crops on this experimental field was under conventional tillage.

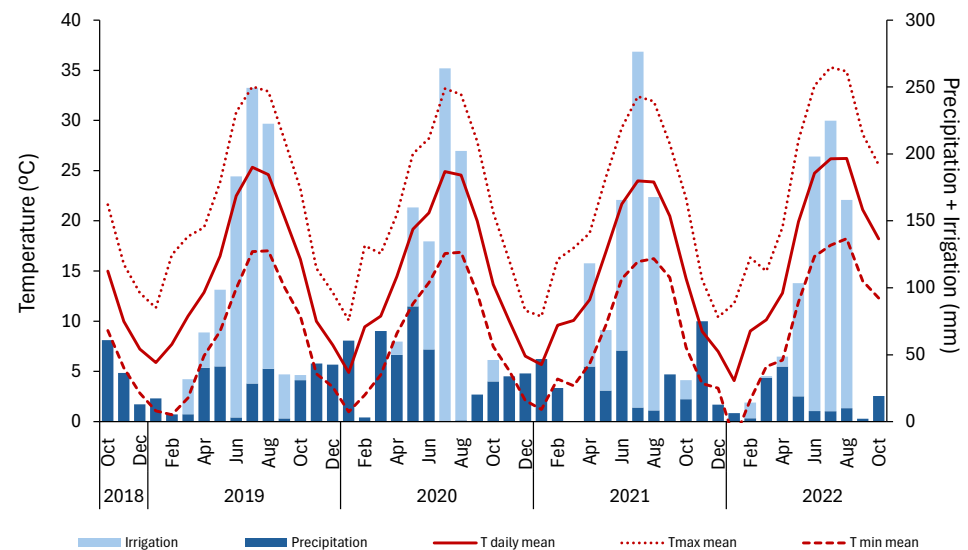


Figure 1. Climatic data. Mean monthly daily mean temperature (solid line), maximum temperature (dotted line) and minimum temperature (dashed line). Blue columns represent monthly precipitation (dark blue) and irrigation (light blue).

In the spring, a long-season maize (*Zea mays*, var. ‘P0933Y’, FAO 500, comparative relative maturity (CRM) of 110) was sown at a sowing rate of 89,000 seeds ha⁻¹ after conventional tillage that incorporated the cover crops into the soil in all the plots. The maize growing season was from April–May to September–October and maize was harvested with a commercial combine and only the grain was removed from the field. Crop sowing and harvest dates can be found in Table 1.

Table 1. Maize and cover crop (CC) sowing and harvest dates, soil mineral nitrogen (SMN) sampling dates, rain and irrigation water applied.

Season	2019		2020		2021		2022	
	CC	Maize	CC	Maize	CC	Maize	CC	Maize
Sowing	18/10/18	10/04/19	21/10/19	16/04/20	09/10/20	20/04/21	14/10/21	08/04/22
Harvest ¹	29/03/19	08/10/19	09/04/20	30/09/20	26/03/21	06/10/21	31/03/22	28/09/22
SMN initial	-	25/03/19	15/10/19	08/04/20	25/09/20	26/03/21	04/10/21	31/03/22
SMN V6	-	07/06/19	-	26/05/20	-	02/06/21	-	02/06/22
SMN final	25/03/19	15/10/19	08/04/20	25/09/20	26/03/21	04/10/21	31/03/22	14/09/22
Rain (mm)	117	160	270	191	172	176	142	88
Irrig. (mm)	26	702	4	627	16	583	14	643

¹ The harvest of the cover crop refers to cover crop termination.

To allow for the use of commercial agricultural machinery, the 2 ha experimental field was divided into two sections. One section was assigned to the NT treatment for establishing the cover crops, whereas the other was assigned to the CT treatment for establishing the cover crops (first main-plot factor). Perpendicular to these treatments, and following a randomized layout, strips corresponding to the cover crop species (second main-plot factor) were established. Each strip was subdivided into two subplots to allocate the two target seeding rates (subplot factor). Thus, the design was considered a strip-split-plot with three replicates for each treatment. The experimental plot size was 3 by 60 m.

The N fertilizer rate was calculated considering maize N uptake for the potential yield of the field (about 12 Mg grain ha⁻¹) and the expected N provided by the legume cover crops. Thus, a total N fertilizer rate of 150 kg N ha⁻¹ was applied in three applications: 50 kg N ha⁻¹ before sowing as a solid fertilizer, and two side-dress applications of 50 kg N ha⁻¹ at the V6 (six leaves with visible collar) and V15 stages with the irrigation water (fertigation) using a UAN-32 solution (50% ureic, 25% nitric, and 25% ammoniacal N). After each fertigation, a low amount of irrigation (7 mm) was applied to incorporate the N to reduce gaseous losses and to avoid leaf burn. A solid complex fertilizer of P₂O₅ and K₂O was applied at a rate of 88 kg ha⁻¹ at pre-planting. In the 2021 and 2022 years, an additional control (without a cover crop) over-fertilized treatment was included in both the CT and NT tillage treatments. In this over-fertilized treatment, the maize received a total N rate of 350 kg N ha⁻¹ to ensure no N limitation (50 at pre-sowing and two 150 kg N ha⁻¹ side-dress applications). Weeds in the maize crop were controlled using herbicides.

Reference ETo was calculated using the FAO Penman–Monteith method [34] with data collected by a nearby weather station over grass. The ETc was calculated by multiplying the daily ETo by the estimated single crop coefficient (kc). Crop coefficients for maize were calculated daily as a function of thermal time using an equation developed at the same location of the experiment [35]. Maize irrigation requirements were determined weekly as the difference between the ETc and the effective precipitation, assumed as 75% of total precipitation. A leaching fraction of 10% was considered to prevent soil salinity build-up due to the salinity of irrigation water (1.5–2 dS m⁻¹). An irrigation efficiency of 90% was considered since most irrigation events were at night to prevent wind-drift and evaporation losses. The cover crops were irrigated (26, 4, 16, and 14 mm year⁻¹ in the 2019, 2020, 2021, and 2022 seasons, respectively) to facilitate crop establishment. The rainfall and irrigation applied can be found in Table 1.

2.3. Cover Crop Measurements

The cover crop plant density (plants m⁻²) was measured one month after sowing in four representative 0.18 m² areas at each experimental unit. The aboveground biomass of the cover crops (2019 to 2022) and weeds (2020 to 2022) were manually sampled before their incorporation into the soil, harvesting two samples of 0.5 m² in each plot. The cover crop and weeds were separated and oven dried at 65 °C, weighed and ground to determine total N and C by combustion (TruSpec CN, LECO, St. Joseph, MI, USA). The percentage of nitrogen derived from atmospheric fixation (Ndfa) in the cover crop was calculated only in treatments with a normal sowing dose, using the natural abundance method [36], according to Equation (1):

$$\%Ndfa = (\delta^{15}N_{RP} - \delta^{15}N_{FL}) \times 100 / (\delta^{15}N_{RP} - B) \quad (1)$$

where the $\delta^{15}N$ of the reference plant (RP) was obtained from the non-leguminous weeds and the $\delta^{15}N$ of the N₂-fixing legume (FL) was obtained from each specific cover crop aboveground biomass. The B value represents the $\delta^{15}N$ of the legume crop growing in a N-free substrate. The B values corresponding to each of the cover crop species were obtained in an adjacent pot experiment in which the three cover crop species were grown using a N-free solution. In the experiment, with four replicates for each cover crop species, seeds from each species were sown in pots in a N-free substrate composed of perlite and coco fibre at 50% each. When the seedlings emerged, they were inoculated at the basal zone with a solution of rhizobia isolated from the same experimental field obtained the previous year.

To produce the rhizobia inoculum for the three species, the following method was used: Firstly, nodules were collected from the roots of the three species and were surface-sterilized

with sterile bidistilled water, ethanol and commercial sodium hypochlorite 3% solution. Secondly, nodule fragments were seeded with a sterile seeding loop in 9 cm diameter plates with King B (KB) medium (Panreac, Barcelona, Spain) and incubated at 25 °C in the dark. Then, single colonies were selected and sub-cultured onto PDA media (Panreac, Barcelona, Spain) to obtain pure cultures of each species. Finally, for the inoculum production, the selected colonies for each species were fermented in liquid culture in Potato Dextrose Broth (PDB) medium (Panreac, Barcelona, Spain) at 25 °C with constant shaking for 4–5 days, to obtain cell suspensions whose concentration was adjusted to 2×10^6 Colony forming units mL^{-1} before being used in subsequent artificial inoculation bioassays.

The pot experiment allowed us to obtain the 'B' values for peas (-0.98 ; $\text{SE} = 0.07$), common vetch (-1.38 ; $\text{SE} = 0.16$), and hairy vetch (-0.33 ; $\text{SE} = 0.08$) adapted to the specific cultivars used in the field experiment. The N uptake by biological nitrogen fixation (BNF) was calculated as the product of the N_{dfa} and the total N contained in the cover crop aboveground biomass.

2.4. Maize Grain Yield and Yield Components

Before harvesting maize with a commercial combine, all maize plants in a subsample of 4.5 m^2 of each plot were manually cut at the ground level. The ears (grain + cobs) were separated from the rest of the plants' aboveground parts and fresh-weighed separately. The number of plants was counted, and the aboveground biomass (except grain + cobs) was fresh-weighed. The ears were threshed to separate the grain from the cobs. A subsample of grain and aboveground biomass was oven dried at 65 °C until constant weight to estimate water content. A subsample of dry aboveground biomass and grain was ground and analyzed by combustion to determine the N and C content (TruSpec CN, Leco, USA). Grain yield (14% water content), thousand kernel weight (TKW), and number of grains per m^2 were determined.

At the maize tasseling stage (VT), leaf greenness was measured at the ear-leaf on thirty plants from each plot using a SPAD-502 chlorophyll meter (KonicaMinolta, Osaka, Japan) to estimate the N sufficiency of the crop.

2.5. Soil Sampling and Analysis

Soil was sampled (0–30 cm depth) at maize harvest (MH) in October, maize sowing (MS) in March–April, and at the maize V6 stage in June to determine the soil mineral nitrogen content (SMN). A 10 g subsample of fresh soil was extracted with 30 mL KCl 2N and nitrate and ammonium concentrations were determined by colorimetry using a continuous-flow analyser (Bran+ Luebbe GmbH, Nordstedt, Germany). Soil samples were dried at 105 °C until constant weight to obtain gravimetric water content. Sampling dates can be found in Table 1.

2.6. Statistical Analysis

The effect of the different factors, cover crop species (S), cover crop sowing type (T), cover crop sowing rate (R), and year (Y), and their interactions were analyzed with analysis of variance considering factor Y as a repeated measure of the experimental plot. All the factors were considered fixed. The analyses were performed using PROC MIXED of the SAS software, version 9.4 (SAS Institute Inc., Cary, NC, USA) according to a strip-split-plot design. When significant differences among treatments were identified at the 0.05 probability level, a mean separation Tukey HSD test was carried out. The results of interactions of S, T and R with Y are given but the separation of means is not provided. The analysis of variance of weed populations, SMN, and maize yield and its components did not include the R factor. The variables were normalized using an optimized Box–Cox transformation when required to comply with the normality and homoscedasticity

conditions. Linear correlations were calculated using Statgraphics Centurion version 18.1.10 software.

3. Results

3.1. Climatic Data

The mean monthly daily temperatures (mean, maximum, and minimum) and cumulative precipitation during the experimental period are shown in Figure 1. The annual mean temperatures were 14.9 °C (2019), 15.2 °C (2020), 14.5 °C (2021) and 15.4 °C (2022). Mean monthly daily minimum temperatures from 0 to −2 °C were observed in January in all years of the study. Mean monthly daily maximum temperatures of 32 to 33 °C were observed in July and August in 2019, 2020 and 2021 and 35 °C in July and August in 2022. Precipitation accumulated during the four cropping seasons (from cover crop sowing in Sep–Oct to maize harvest) and during the intercrop (IC) and maize (M) periods was 277 mm (IC = 117; M = 160), 460 mm (IC = 270; M = 191), 338 mm (IC = 172; M = 176), and 230 mm (IC = 142; M = 88) in 2019 to 2022, respectively.

3.2. Cover Crops

3.2.1. Establishment

Cover crop plant density (Table 2) was significantly affected by the cover crop species, sowing type, sowing rate and year. The T × Y and S × Y interactions were significant. The cover crop plant density was 19% higher with CT compared to NT. At a normal cover crop sowing rate, the plant density was 16% higher than with a reduced sowing rate. The highest cover crop plant density was observed in common vetch and the lowest in pea, with hairy vetch showing intermediate values. Differences in cover crop plant density were observed among years, ranging from 59 plants m^{−2} in 2022 to 93 plants m^{−2} in 2019.

Table 2. The effect of cover crop species (S), cover crop sowing type (T), cover crop sowing rate (R) and year (Y) on cover crop plant density, biomass, N uptake and N content. For each variable, values followed by different letters are significantly different ($p < 0.05$) after ANOVA according to a Tukey test.

Factor	Level	Plant Density n° m ^{−2}	Biomass kg ha ^{−1}	N Uptake kg ha ^{−1}	N Content %
S	Pea	70.5 b	2430 a	74.8 a	3.14 c
	Common vetch	83.1 a	2180 a	80.0 a	3.69 b
	Hairy vetch	78.5 ab	1306 b	50.0 b	3.84 a
T	CT	83.9 a	2007	69.2	3.54
	NT	70.8 b	1937	67.3	3.57
R	Normal	83.0 a	2058 a	71.3 a	3.57
	Reduced	71.8 b	1886 b	65.2 b	3.55
Y	2019	92.8 a	1764 c	59.3 c	3.50 b
	2020	71.0 b	2740 a	94.5 a	3.52 b
	2021	87.2 a	2355 b	80.7 b	3.47 b
	2022	58.6 c	1029 d	38.5 d	3.74 a
	CT-Normal	-	2023 a	70.0 a	-
	CT-Reduced	-	1991 ab	68.4 ab	-
	NT-Normal	-	2094 a	72.6 a	-
	NT-Reduced	-	1780 b	62.0 b	-
	CT-Pea	-	2324 a	69.2 b	3.060 c
	CT-Common vetch	-	2298 a	85.3 a	3.76 ab

Table 2. Cont.

Factor	Level	Plant Density n° m ⁻²	Biomass kg ha ⁻¹	N Uptake kg ha ⁻¹	N Content %
	CT-Hairy vetch	-	1399 a	53.0 c	3.82 ab
	NT-Pea	-	2536 a	80.4 ab	3.22 c
	NT-Common vetch	-	2062 a	74.6 ab	3.63 b
	NT-Hairy vetch	-	1212 b	46.9 c	3.87 a
ANOVA (<i>p</i> -values)					
	Species (S)	0.002	<0.001	<0.001	<0.001
	Sowing type (T)	<0.001	0.163	0.266	0.84
	Sowing rate (R)	<0.001	0.015	0.011	0.514
	Year (Y)	<0.001	<0.001	<0.001	<0.001
	S × R	0.639	0.573	0.591	0.257
	S × Y	<0.001	<0.001	<0.001	0.011
	T × R	0.100	0.037	0.040	0.648
	T × S	0.404	0.032	0.005	0.038
	T × Y	<0.001	<0.001	<0.001	0.034
	R × Y	0.629	0.016	0.005	0.259
	L × S × Y	0.004	0.142	0.178	0.613
	L × S × R × Y	0.313	0.248	0.170	0.190

Abbreviations: CT = conventional tillage; NT = no-tillage.

3.2.2. Biomass and N Content

Cover crop biomass (Table 2) was significantly affected by the cover crop species, sowing rate, and year. The interactions T × R and T × S were also significant. All the double interactions with the factor year (Y) were significant. The biomasses of pea and common vetch were 76% higher than the hairy vetch biomass. Cover crop biomass under the normal sowing rate was 9% higher than under the reduced rate. Differences were observed among years, with the highest value observed in 2020 and the lowest in 2022. Lower cover crop biomass (−14%) was produced with the reduced sowing rate when the cover crop was sown without tillage. Considering the data from the four-year period, a significant correlation was observed between cover crop biomass and plant density in pea ($r = 0.51$; p -value <0.001) but not in the vetch species.

The N uptake of the cover crops was significantly affected by the cover crop species, sowing rate and year. The T × R and T × S interactions were significant. The N uptake of the cover crops was strongly related to biomass production in all cover crop species (pea: $r = 0.98$, p -value < 0.001; common vetch: $r = 0.98$, p -value < 0.001; hairy vetch: $r = 0.99$, p -value <0.001). Due to this strong correlation, the differences observed for N uptake followed the same pattern as those observed for biomass. The N uptake in pea and common vetch was 50–60% higher compared to hairy vetch. The T × S interaction showed that N uptake under conventional tillage was higher in common vetch than in pea but not under no-tillage. N uptake varied significantly from year to year, associated with differences in total biomass, ranging from 38.5 to 94.5 kg N ha⁻¹.

The N content of cover crop biomass (Table 2) was affected by the cover crop species and the year. The interactions T × S, T × Y, and S × Y were also significant. Hairy vetch had the highest N content and pea the lowest, with the common vetch producing an intermediate value. Differences in the N content of cover crop biomass between the experimental years were significant, with the highest value in 2022, when the biomass production was lower. The N content of hairy vetch was higher than that of common vetch when the cover crops were sown without previous tillage.

3.2.3. Nitrogen Derived from Biological Fixation (Ndfa and BNF)

The proportion of nitrogen derived from the atmosphere in the legume cover crops (Ndfa) (Table 2) was affected by the cover crop species, sowing type, and year. The $T \times Y$ and $S \times Y$ interactions were significant. The Ndfa was 17% higher when cover crops were sown after tillage. Common vetch and pea showed higher Ndfa values compared to hairy vetch. High interannual differences were observed, with the highest Ndfa value in 2021 and the lowest in 2019.

The N uptake derived from biological nitrogen fixation (BNF) (Table 3) was strongly correlated with the cover crop aboveground biomass in all the species (Pea: $r = 0.813$, p -value < 0.001 ; common vetch: $r = 0.952$, p -value < 0.001 ; hairy vetch: $r = 0.688$, p -value < 0.001). The BNF was affected by the cover crop species, sowing type and year. The $S \times Y$ interaction was also significant. The BNF was 14% higher when cover crops were sown after conventional tillage. The BNF was higher in pea and common vetch in comparison with hairy vetch.

Table 3. The effect of cover crop species (S), cover crop sowing type (T) and year (Y) on the proportion of nitrogen derived from the atmosphere (Ndfa) and the total nitrogen derived from biological fixation (BNF). For each variable, values followed by different letters are significantly different ($p < 0.05$) after ANOVA according to a Tukey test.

Factor	Level	Ndfa %	BNF kg N ha ⁻¹
S	Pea	77.7 ab	60.8 a
	Common vetch	82.0 a	69.6 a
	Hairy vetch	73.0 b	37.1 b
T	CT	83.5 a	59.7 a
	NT	71.6 b	52.4 b
Y	2019	54.6 d	36.4 b
	2020	78.8 c	70.3 a
	2021	90.7 a	80.7 a
	2022	85.2 b	36.0 b
ANOVA (p -values)			
	Species (S)	0.040	<0.001
	Sowing type (T)	<0.001	0.030
	Year (Y)	<0.001	<0.001
	$S \times T$	0.800	0.051
	$S \times Y$	<0.001	<0.001
	$T \times Y$	<0.001	0.055
	$L \times S \times Y$	0.553	0.140

Abbreviations: CT = conventional tillage; NT = no-tillage.

3.2.4. Interaction of Cover Crop Biomass and Weed Biomass

Considerable weed growth was observed during the cover crop growth period, both in the plots sown with cover crops and in the control plots without cover crops. The weed biomass was affected by the cover crop species, cover crop sowing type, and year (Table 4). The $T \times Y$ and $S \times Y$ interactions were significant. The weed biomass was 44% higher when cover crops were sown after no-tillage than after conventional tillage. The weed biomass was reduced at least by half in treatments where a cover crop was sown compared to the control treatment. Significant differences in weed biomass between years were observed, with lower values in 2022 compared to the previous years. Significant negative correlations were observed between cover crop and weed biomass in 2020 ($r = -0.33$; $p = 0.04$) and 2021 ($r = -0.56$; $p < 0.001$).

Table 4. The effect of cover crop (CC) species (S), cover crop sowing type (T), and year (Y) on weed biomass, weed + cover crop biomass, and N uptake of weeds + cover crop. The ratio C/N of the weed + cover crop biomass is also presented. For each variable, values followed by different letters are significantly different ($p < 0.05$) after ANOVA according to a Tukey test.

Factor	Level	Weed Biomass kg ha ⁻¹	Weed + CC Biomass kg ha ⁻¹	Weed + CC N Uptake kg ha ⁻¹	Weed + CC C:N
S	Pea	1175 b	3518 a	100 a	15.4 b
	Common vetch	1053 b	3428 a	113 a	13.4 c
	Hairy vetch	1264 b	2669 b	84.6 b	14.1 c
	Control	2413 a	2413 b	59.5 c	19.1 a
T	CT	1138 b	2936 b	91.1 b	14.8 b
	NT	1533 a	3361 a	96.7 a	15.1 a
Y	2020	1498 a	3847 a	121 a	14.2 b
	2021	1588 a	3656 a	108 b	15.4 a
	2022	921 b	1803 b	52.9 c	15.3 a
	CT-Pea	-	3280 ab	93.1 b	-
	CT-Common vetch	-	3564 a	121 a	-
	CT-Hairy vetch	-	2509 c	81.6 b	-
	CT-Control	-	1844 d	45.7 c	-
	NT-Pea	-	3755 a	107 ab	-
	NT-Common vetch	-	3292 ab	105 ab	-
	NT-Hairy vetch	-	2829 bc	87.7 b	-
	NT-Control	-	3054 abc	75.0 b	-
ANOVA (<i>p</i> -values)					
	Species (S)	<0.001	<0.001	<0.001	<0.001
	Sowing type (T)	<0.001	<0.001	0.004	0.039
	Year (Y)	<0.001	<0.001	<0.001	<0.001
	T × S	0.344	<0.001	<0.001	0.412
	S × Y	0.035	<0.001	0.034	0.019
	T × Y	<0.001	<0.001	<0.001	0.014
	T × S × Y	0.214	0.004	0.014	0.131

Abbreviations: CT = conventional tillage, NT = no-tillage, and CC = cover crop.

The weed plus cover crop biomass was affected by the cover crop species, cover crop sowing type, and year. All the interactions were significant. The weed plus cover crop biomass was 14% higher in NT plots and was higher (+29%) in the pea and common vetch treatments compared to the hairy vetch and control (only weeds) treatments. The weed plus cover crop biomass in 2022 was lower than the previous years. The T × S interaction shows that the weed plus cover crop biomass with NT was 66% higher than under conventional tillage in the control treatment (only weeds), but no effect was observed when a cover crop was sown.

The N uptake of the weeds plus cover crops was affected by the cover crop species, cover crop sowing type, and year. T × S, T × Y, and S × Y interactions were significant. The N uptake of the weeds plus cover crops was slightly higher under no-tillage than under conventional tillage treatment. The N uptake of the weeds plus cover crops of the common vetch and pea treatments was higher than that of the hairy vetch and more than 60% higher than the control treatment. The T × S interaction show that the N uptake of the weeds plus cover crops in the common vetch treatment was higher than all the other treatments when the cover crops were sown with previous tillage, but it was not significantly different when common vetch was sown without previous tillage.

The C:N ratio of the weed plus cover biomass was affected by the cover crop species, cover crop sowing type, and year (Table 4). The $T \times Y$ and $S \times Y$ interactions were significant. The C:N ratio was lower when vetch species were used as cover crops, and it was higher in the control treatment (only weeds). The pea treatment showed an intermediate value of C:N between the vetch species and the control treatments. Some minor differences were observed among years.

3.3. Soil Mineral Nitrogen

SMN before CC incorporation was affected by the cover crop sowing type and the year but not by the cover crop species (Table 5). The SMN before CC incorporation was low, but it was higher ($+7 \text{ kg ha}^{-1}$) when the cover crops were sown without previous tillage compared to when they were sown after conventional tillage. The SMN changed significantly over the four experimental seasons, ranging from 11 to 40 kg ha^{-1} .

Table 5. The effect of cover crop species (S), cover crop sowing type (T), and year (Y) on soil mineral nitrogen in the upper part of the soil profile (0–30 cm depth) before cover crop incorporation, at the maize V6 stage and at maize harvest. For each variable, values followed by different letters are significantly different ($p < 0.05$) after ANOVA according to a Tukey test.

Factor	Level	Before CC Incorporation	Maize V6 kg N ha ⁻¹	Maize Harvest
S	Pea	19.7	162 a	45.3 ab
	Common vetch	21.1	168 a	48.1 a
	Hairy vetch	21.1	171 a	42.0 ab
	Control	17.5	121 b	31.9 b
T	CT	16.7 b	156	39.9 b
	NT	23.7 a	165	46.5 a
Y	2019	40.1 a	188 a	31.2 b
	2020	18.2 b	144 b	53.9 a
	2021	11.4 c	194 a	30.7 b
	2022	11.0 c	117 c	57.1 a
ANOVA (<i>p</i> -values)				
	Species (S)	0.119	0.004	0.029
	Sowing type (T)	0.003	0.236	0.037
	Year (Y)	<0.001	<0.001	<0.001
	S × T	0.578	0.247	0.764
	S × Y	0.052	0.043	0.516
	T × Y	0.141	0.213	0.019
	T × S × Y	0.643	0.035	0.238

Abbreviations: CT = conventional tillage, NT = no-tillage, and CC = cover crop.

The SMN at the maize V6 stage was affected by the cover crop species and the year (Table 5). The $S \times Y$ interaction was also significant. The SMN at the maize V6 stage was lower (−28%) in the control treatment compared to the cover crop treatments, but no differences were observed among the three cover crop species. Considering the four years of the study, a significant positive correlation ($r = 0.407$; $p < 0.001$) was observed between the N uptake of the weeds plus cover crops and the SMN at the maize V6 stage.

Soil mineral nitrogen content (0–30 cm) at maize harvest was affected by the cover crop species, cover crop sowing type, and year (Table 5). The $T \times Y$ interaction was significant. When the cover crops were sown without previous tillage, the SMN at maize harvest was 17% higher than when they were sown with previous tillage. Some differences were observed between cover crop treatments, with lower SMN at maize harvest in the control

(−34%) compared to the common vetch treatment but without differences among the three cover crops evaluated. Some differences in SMN at maize harvest were observed between the four years of the study.

3.4. Maize Yield, Yield Components and N Uptake

The maize plant density at harvest was affected by the cover crop species and the year (Table 6). The T × Y interaction was also significant. Maize plant density was slightly higher (+4%) in the common vetch treatment compared to the control treatment, but no differences were observed among the other treatments.

Table 6. The effect of cover crop species (S), cover crop sowing type (T), and year (Y) on the maize plant density, grain yield (14% humidity) and yield components (thousand-grain weight: TGW) and number of grains per m^{−2}. Maize grain N content and N uptake and the chlorophyll meter readings of leaves at the VT (tasseling) stage (SPAD) are also presented. For each variable, values followed by different letters are significantly different ($p < 0.05$) after ANOVA according to a Tukey test.

Factor	Level	Plant Density Plants ha ^{−1}	Grain Yield Mg ha ^{−1}	N kg ha ^{−1}	TGW g	N° Grain m ² Grain m ^{−2}	Grain N %	SPAD VT
S	Pea	80,309 ab	12.3 a	222 a	273	3852 a	1.36 a	52.8 ab
	Common vetch	81,327 a	12.7 a	234 a	274	3991 a	1.36 a	53.1 a
	Hairy vetch	80,602 ab	12.5 a	227 a	274	3901 a	1.36 a	52.7 ab
	Control	77,963 b	11.2 b	195 b	268	3574 b	1.32 b	51.8 b
T	CT	79,656	12.1	222	271 b	3841	1.37	52.5
	NT	81,041	12.4	224	275 a	3892	1.34	52.8
Y	2019	79,453 ab	12.3 b	237 a	283 b	3741 b	1.41 a	54.1 a
	2020	76,455 c	13.0 a	242 a	293 a	3828 b	1.44 a	53.9 a
	2021	85,732 a	13.7 a	220 b	277 c	4266 a	1.24 c	50.0 c
	2022	79,753 b	10.1 c	193 c	239 d	3630 c	1.33 b	52.7 b
ANOVA (<i>p</i> -values)								
Species (S)		0.015	<0.001	<0.001	0.091	0.001	0.025	0.037
Sowing type (T)		0.067	0.056	0.464	0.009	0.466	0.129	0.284
Year (Y)		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
S × T		0.524	0.170	0.187	0.134	0.468	0.133	0.408
S × Y		0.069	0.009	0.076	0.049	0.078	0.126	0.487
T × Y		0.025	0.055	0.634	0.519	0.007	0.201	0.008
T × S × Y		0.219	0.741	0.28	0.362	0.450	0.807	0.510

Abbreviations: CT = conventional tillage; NT = no-tillage.

Grain yield was affected by the cover crop species and the year. The T × Y and S × Y interactions were also significant. Maize yield increased around 12% when a cover crop was grown as compared to the control treatment. Differences in grain yield were observed among years, with the lowest yield in 2022 and the highest yield in 2020 and 2021.

The thousand-grain weight (TGW) of maize was affected by the cover crop sowing type and the year (Table 6). Cover crop sowing without previous tillage slightly increased the TGW of maize compared to the cover crop sowing with previous tillage. The TGW ranged from 239 g to 293 g (+23%) between years. The number of grains per m² (Table 6) was affected by the cover crop species, the year and the S × Y interaction. The introduction of legume winter cover crops increased the number of grains per m² by 12% compared with the control treatment. The number of grains per m^{−2} was higher in 2021, and the

lowest value was in 2022. The SPAD values at the maize VT stage were affected by the cover crop species and the year (Table 6). A significantly higher SPAD value was observed in maize leaves after the common vetch cover crop compared to the control treatment, without differences among the other pair-wise comparisons. Significant differences were also observed among years.

The maize N uptake was affected by the cover crop species and the year. The maize N uptake was higher (+17%) when grown in the cover crop treatments compared to the control treatment and was associated with higher grain yield and N grain content. In 2021 and 2022, when a N over-fertilized control treatment was included in the experiment, no differences were found in the two-year average of grain yield between the over-fertilized control and the cover crop treatments (Figure 2).

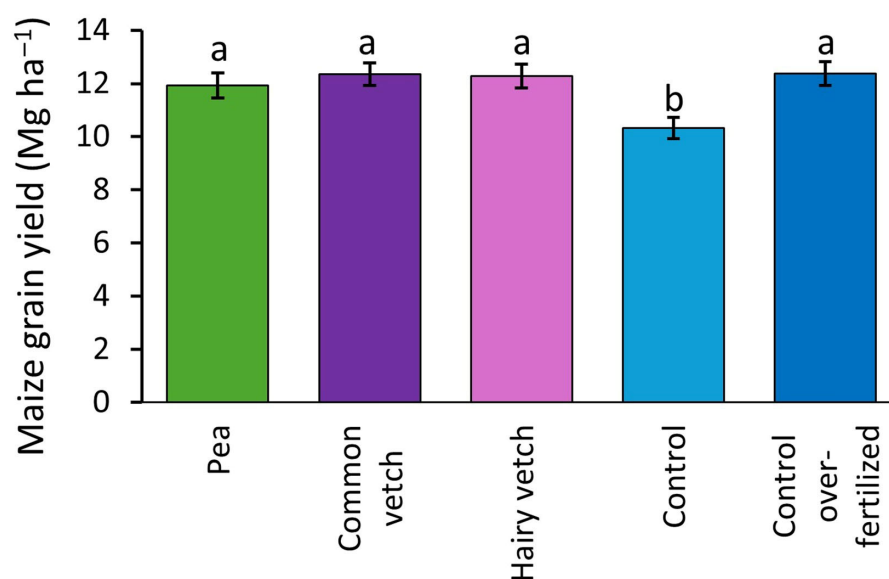


Figure 2. Two year (2021 and 2022) average of maize grain yield (14% humidity) after the different cover crop species treatments (pea, common vetch, hairy vetch, and control) and the over-fertilized control treatment (350 kg N ha⁻¹). Values followed by different letters are significantly different ($p < 0.05$) after ANOVA according to a Tukey test.

4. Discussion

4.1. Cover Crop Development

The reduction in the cover crop plant density when sown without previous tillage compared to when sown with previous conventional tillage was related to the presence of a high amount of maize crop residues, which makes adequate seed placement and emergence under no-tillage conditions more difficult [37]. However, the lower plant density in the no-tillage treatment did not affect cover crop biomass production, which may be attributed in part to compensatory growth, allowing for similar aboveground biomass production despite lower initial establishment. In farmers' fields, tillage may delay the sowing of cover crops, resulting in a reduction in the final biomass [11], although in the present study, cover crops were sown the same day in both sowing methods. On average for all the cover crops, the implementation of a 25% reduction in the cover crop sowing rate dose reduced its plant density by 16% compared to the normal sowing rate. However, the reduction in plant density only resulted in a reduction in cover crop biomass when the cover crops were sown without previous tillage. The correlation between cover crop plant density and biomass was only significant in pea, probably due to the lower plant density observed compared to the vetch species.

Vetch species are the legumes most commonly used as a cover crop due to their wide adaptability [38]. Studies conducted under similar climatic and management conditions [1,20] report biomass production for hairy and common vetch similar to the biomass obtained in this study for pea and common vetch. However, hairy vetch had lower biomass development, probably due to its longer development cycle potential [39] more adapted to cold climates, which prevented this species from reaching its full growth under the climatic conditions of the experiment. Nevertheless, it should be noted that in [1], hairy vetch biomass widely ranged from 576 to 5055 kg ha⁻¹ with a similar growing season (October–March), suggesting that differences can also be attributed to the crop cultivars used in the study. Large differences in cover crop biomass production between years were related to variability in climatic conditions during the experimental period [40]. Differences in rainfall and occasional irrigation during the cover crop period may have favoured the growth of cover crops in early spring in 2020 and 2021, growing on average 88 and 52% more than in 2019 and 2022, respectively. In addition, differences in biomass production in 2019 and 2022 could be related to the difference in the average minimum temperatures reached in January in 2019 (1.08 °C) and 2022 (−1.84 °C), which affected negatively the growth of the cover crops. Due to the late sowing of some cover crop species such as vetch in October compared to the optimal sowing date in the region (September), the winter frost events can have negative consequences on the development of these species, limiting their success as cover crops. As indicated by [41], the maximum benefits of leguminous cover crops in cold areas are obtained by early sowing. Although the pea is less frequently considered as a potential cover crop species, the results indicate that under the climatic and crop management conditions of the study, the pea can perform similarly to common vetch in terms of total aboveground biomass and fixed N, with the advantage of a higher frost tolerance, which can be relevant in the case of late sowing dates of the cover crop. Under more humid conditions in the Pampean region [42], reported wide biomass production for pea and common vetch ranges from 676 to 7145 and from 1925 to 11,784 kg ha⁻¹, respectively. However, in semi-arid temperate zones, [43] reported a similar biomass production for pea (approx. 2500 kg ha⁻¹) to those observed in this study.

The N uptake by the cover crops was strongly correlated with biomass production. According to [2], the N uptake of leguminous cover crops ranges from 50 to 300 kg N ha⁻¹, which is consistent with the results obtained for the three tested species. The N uptake for common vetch is also consistent with that reported [20] in the same region (62 kg N ha⁻¹), whereas the N uptake for hairy vetch in this study is lower than reported in the central part of Spain (85 kg N ha⁻¹) [1]. The N uptake of peas was similar to common vetch, as the lower N concentration of peas was compensated by its higher aboveground biomass production.

4.2. Biological Nitrogen Fixation of Cover Crops

The capacity for biological nitrogen fixation of legumes depends not only on plant–rhizobium interaction but also on environmental factors such as soil acidity, salinity, temperature, moisture content and nutrient availability [25,26,44]. The Ndfa values reported [45] for pea (68%) and for common vetch (64%) [46] are lower than those observed in this study for both species. Ref. [25] reported a wide range for Ndfa values for pea in Europe (26–99%), close to the range observed in this study (47–95%). This highlights the variability in biological nitrogen fixation depending on environmental conditions.

High SMN can reduce or inhibit biological N fixation [25,47]. In general, the Ndfa values in this study exceeded the 60% threshold considered as high by [48]. However, these authors reported Ndfa values over 60% in soils with SMN values (0–90 cm soil depth) lower than 50 kg N ha⁻¹, while in this study, the SMN at cover crop sowing was relatively high, as 74% of the values were between 55 and 201 kg N ha⁻¹ (0–0.30 m soil depth). The

cover crop sowing type slightly affected cover crop Ndfa values in this study, contrary to results reported for faba bean, where tillage does not affect Ndfa [49]. Although increased rates of N mineralization could be expected to reduce the Ndfa values after conventional tillage, the high C:N ratio (54–67) of maize crop residues can lead to N immobilization during the cover crop growing period [50,51], which could reduce differences in SMN and the effect on the nitrogen fixation capacity. As observed in previous studies [42,48], when high Ndfa values are observed, the differences between species in total N mass derived from biological fixation (BNF) are mostly related to differences in cover crop biomass.

4.3. Weeds and Cover Crops

Cover crops have been proposed as a fundamental component of integrated control of weed growth in crops [52]. Weed reduction in intercropping periods with cover crops is often associated with the speed and amount of biomass produced by the cover crops [53], as was partially observed in some years of this study (2020 and 2021) but not in 2022, when both cover crop and weed biomass were significantly reduced compared to the other years. The conventional tillage used for cover crop sowing reduced weed growth at the maize planting time, particularly in the control treatment. Although it was not assessed in this study, Refs. [54,55] reported that the introduction of leguminous species can reduce weed growth during the summer crop period, which could imply an additional benefit of the introduction of winter legume cover crops in maize systems.

The cover crop biomass ranged between 50 and 70% of the total biomass (cover crop plus weeds) growth and between 56 and 78% of the total N uptake. Thus, the growth of weeds did not affect significantly the C:N ratio or N uptake in treatments where cover crops were included. The weeds that grew in the control treatment were mainly non-leguminous species, and in the no-tillage treatment produced a similar biomass of the sum of weeds and cover crops. However, the C:N ratio in the control treatment (only weeds) was higher than in the treatments with cover crops, perhaps causing N immobilization compared to the legume cover crop treatments.

4.4. Evolution of Soil Mineral Nitrogen

Similarly to results described in other studies [56,57], the lowest SMN was observed at the end of the intercropping period due to the combined N uptake of the cover crops and weeds. Thus, the leguminous cover crops decreased the SMN in the topsoil in a similar way to the weeds that grew in the control plots. The main objective of the inclusion of leguminous winter cover crops, besides reducing nitrate leaching [58], is to provide additional N to the system, allowing for reductions in N fertilizer in the subsequent maize crop. Previous studies [2] report that, after a leguminous cover crop, the estimated reductions in N fertilizer for subsequent crops ranges from 33% to 50% of the N uptake of the cover crop. The higher N uptake in the treatments with leguminous cover crops, and their low C:N ratio (C:N = 13.4–15.4), probably promoted high N mineralisation [51] compared to the control plots, which included only weeds and had a higher C:N ratio (19.1).

4.5. Cover Crop Effects on Maize Yield

Maize sown after the common vetch cover crop had a slightly higher plant density at harvest compared to the control treatment. This is contrary to other studies [55] that found that cover crops generally reduce maize emergence rates, although they found that the reduction was lower after leguminous cover crops.

Contradictory results have been reported regarding the effect of leguminous cover crops on the subsequent cash crop yield [59]. Some studies [10,20,60] reported no effect or even yield penalties. However, the advantages of leguminous cover crops have been documented when SMN is low [59,61]. In this study, where low N fertilizer rates were

applied (150 kg N ha^{-1}), maize yield increased by 12% after leguminous cover crops compared to the control treatment. Moreover, the introduction of leguminous cover crops allowed us to reach maize yields similar to those reported in maize crop systems [18,62] in the Ebro valley ($12 \text{ to } 17 \text{ Mg ha}^{-1}$). Similarly to what was found by [57], the cover crop sowing type did not affect maize yield.

The cover crops' effect on the following cash crop depends on the synchronization of the N mineralization after their incorporation with the following crop's N requirements [40,50,63]. In maize, the highest N uptake is concentrated during a short period of time between the V6 stage and tasselling. During this period, N demand increases exponentially in line with vegetative development [64]. The higher N availability at the V6 stage observed in this study in the leguminous cover crop treatments, as well as the positive correlation between SMN at the V6 stage and the final maize grain yield, suggest a favourable impact of a N surplus when a leguminous cover crop is grown. This was reflected in the increase of 12% of maize yield in treatments where leguminous cover crops were grown compared to the control treatment. According to a study under similar agronomic and climatic conditions [65], the leaf SPAD values observed in the control treatment could indicate suboptimal levels of N availability for maize, which can affect carbon assimilation. This decrease in carbon assimilation, resulting from lower N availability, has a significant impact on the number of grains per m^{-2} [66], as was observed in this study in the common vetch cover crop treatment compared to the control, although this was not significant with the other cover crop species.

The introduction of leguminous cover crops increased the N content of maize grains and the N uptake in total biomass after the first experimental year, whereas other studies reported this effect only after multiple subsequent cropping seasons [1]. This is probably related to the low N fertilizer rates used in our study. The lower average grain N content observed in 2021 compared to the other growing seasons coincided with the year of highest grain production, which can be explained by the dilution effect described in other studies [67].

According to the results of this study, the N mineralisation from leguminous cover crop residues seemed synchronized with the N requirements of maize, improving its yield and N uptake content compared to maize growth after winter fallow. However, further studies to increase understanding of the temporal release of N from cover crop residues under different management and environmental conditions are necessary to properly adjust N fertilizer applications to maize crop in order to improve the NUE and encourage the adoption of legume cover crops by farmers.

5. Conclusions

The introduction of legume cover crops during the autumn–winter intercropping period increased maize yields compared to the fallow system with only weed growth, regardless of the sowing type, sowing rate and cover crop species. The legume cover crops provided a considerable input of N to the soil derived from biological fixation, which allowed for a significant reduction in the maize N fertilizer. Under the environmental and crop management conditions of this investigation, common vetch and pea produced a greater biomass and contributed more total N to the system than hairy vetch, although the final effect on maize yield was similar among the three evaluated species. It is possible to increase the use of leguminous cover crops by sowing with no-tillage and reducing the standard sowing rates by 25% in maize-based cropping systems, given that the N uptake of the cover crops and maize grain yield were not affected. These findings support the use of legume cover crops as a strategy to improve N use efficiency and with potential to reduce the environmental impact of irrigated maize systems.

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