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Maize yield and N dynamics after cover crops introduction

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ABSTRACT

The use of cover crops (CCs) is widely suggested as a sustainable agricultural practice. Nevertheless, conflicting results have been reported about the short-term effect of CCs on cash crop yields and the soil nitrogen (N) dynamics. Within this framework, the present study aims to examine the short-term impact of CC introduction into a conventional agricultural system on silage maize yield and the N dynamics (maize N uptake, N use efficiency (NUE), soil nitrate content (Nmin), and apparent soil N mineralization and immobilization processes) in northern Italy. The CC systems (~5.5 ha) included a fixed treatment (FI) with a gramineous species (triticale), a 2-year gramineous-legume species succession (SU) (rye, clover), and a weed-covered control treatment (NoCC). In the first year, triticale and rye had the same total (aboveground + root) final biomass (2.5 Mg ha^{-1} on average), C:N ratio (29), and N uptake (36.4 kg ha^{-1}). However, triticale developed faster in the first winter months. Both grass species equally reduced the soil Nmin content over the winter season (as valid catch crops), but they caused apparent N immobilization during the following maize growing season. In the second year, clover produced the same total biomass as triticale did (1.8 Mg ha^{-1}), but with a higher total N content (72.5 kg ha^{-1}) and lower C:N ratio (27) which determined a lower apparent N immobilization. The introduction of CCs did not affect the yield of maize. During the maize growing season, lower N uptake and NUE were recorded after CCs grasses species cultivation compared to clover and NoCC. These observations suggest that a key aspect to be considered when dealing with CCs is understanding the N mineralization-immobilization processes related to CC residue decomposition, which might determine N availability for the subsequent crop and in turn its production quality (N uptake), even when the yield is not affected.

1. Introduction

The use of cover crops (CCs) is becoming a viable option to improve agricultural sustainability in the context of climate change (Blanco-Canqui et al., 2015). They can improve soil properties by affecting its fertility, and especially the nitrogen (N) cycle dynamics (Scavo et al., 2022), while enhancing or maintaining crop yields. Nevertheless, broad variations of cash crop yield response to CC have been reported in previous reviews and meta-analyses (Tonitto et al., 2006; Blanco-Canqui et al., 2015; Marcillo and Miguez, 2017; Ruis and Blanco-Canqui, 2017; Daryanto et al., 2018; Abdalla et al., 2019). Understanding the repercussions of winter CCs on the final cash crop yield is a key point for adopting CCs (Singer et al., 2007) that needs to be investigated considering that it can be affected by many factors such as the region, the cash and CC species, climate conditions, and agricultural management. Looking at maize crop, in Italy, previous studies observed that both yield crop and N uptake were significantly affected by winter CC introduction. Testing different CC species, Caporali et al. (2004) observed higher maize yield following legumes compared to grasses and weed-covered control. Coupling the CC introduction with reduced or no-tillage management, Boselli et al. (2020) showed that CCs were effective in enhancing soil fertility in the Po Valley (Northern Italy), without reducing maize yield in both tillage systems. Nevertheless, in the same area, Fiorini et al. (2022) registered an initial lower maize yield after 2 years of winter CCs and no effect after the third year. Moreover, studies conducted in both Northern and Central Italy underlined the effect of winter CCs on soil N cycling (especially in the 0–30 cm layer), with increased soil total N content registered after CC introduction (Mazzoncini et al., 2011; Boselli et al., 2020).

CC adoption has been recently promoted by the new European Common Agricultural Policy (CAP) 2023–2027 (https://agriculture.ec.europa.eu/common-agricultural-policy/cap-overview/cap-2023–27_en). Within this framework, it appears of utmost interest to conduct field trials to investigate different CCs so as to maximize the

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beneficial effects of this practice for land managers and minimize the risk of cash crop yield reduction.

Similarly to what was observed in Italy, it has been reported that leguminous CCs – especially crimson clover – increase maize yield and N uptake in other parts of the world (Kramberger et al., 2014). Inversely, maize yield reduction and lower N uptake have often been observed shortly after non leguminous CC species (Tonitto et al., 2006; Kramberger et al., 2009; Gabriel et al., 2013). However, winter annual grasses, characterized by high N uptake capabilities and NUE (Ketterings et al., 2015), are usually suggested as CCs after high N input crop cycles to reduce N leaching risk and control weeds (Mergoum et al., 2009).

Regardless of the species, the short-term effects of CCs can be masked by conventional N fertilization practices at non-limiting doses (Bundy and Andraski, 2005; Miguez and Bollero, 2006; Marcillo and Miguez, 2017). Other studies report that leguminous and grass CCs can increase maize yield, while leguminous CCs can also improve NUE only when N fertilizer application is below the N requirements of the crop (Bundy and Andraski, 2005; Gabriel and Quemada, 2011). In addition, the variability of climate conditions can change the effect of CCs on following cash crops even in two subsequent years on the same location (Hashemi et al., 2013). Under cold and dry conditions, grasses usually outperform leguminous CCs, and are also more effective catch crops (Ramírez-García et al., 2015), but the opposite has been reported under dry and low mineral N availability conditions (Brychkova et al., 2022).

The high potential variability of CC performances and their effect on cash crop production, especially in the first years following introduction, often hinders CC adoption in conventional agricultural systems worldwide as well as in Italy. Within this framework, studying the short-term performance of different CC species is of utmost importance to analyze their controversial effects on cash crop yield and the N dynamics. In addition, trying to understand the potential effect of CC residues on apparent N mineralization and immobilization processes is crucial to compute a preliminary investigation of the effects of organic residue incorporation on soil N cycling (Quemada and Cabrera, 1997; Cabrera et al., 2005).

The objective of this study was to examine the short-term effect of replacing fallow periods with two CC species successions (grassess followed by grasses; grassess followed by a leguminous species) on silage maize production in a loamy soil under humid subtropical climate conditions. The analysis included the investigation of the effects of CCs on maize yield and the N dynamics (maize N uptake, NUE, soil nitrate (Nmin) content, and apparent soil N mineralization and immobilization processes).

2. Materials and methods

2.1. Site description

The research was conducted in the experimental farm "L. Toniolo" of the University of Padova (45°20′53″ N, 11°57′11″ E, 6 m above sea level). The farm was located in a plain of fluvial origin in Northeastern Italy. Water table fluctuating from 0.5 to 1.5 m in late winter-early spring to 1.5–3 m in summer. The area fell within the Cfa class of the Köppen classification (Rubel et al., 2017), with rainfall mainly concentrated in the spring and autumn months, and frequent thunderstorms in hot-humid summers. Climate data (1994–2019) collected from the Regional Agency for Environmental Protection (ARPAV) were 841 mm annual rainfall and an average annual temperature of 13.6 °C (with average minimum and maximum temperatures of 8.9 and 18.7 °C, respectively). The soil of the experimental site was characterized by a loamy texture (Schoeneberger et al., 2012) and classified as Fluvi-Calcaric Cambisol (CMcf) (FAO-UNESCO, 1990) with a high carbonate content (32%). The main physical and chemical characteristics of the topsoil layer (0–40 cm) are listed in Table 1.

Table 1
Physical-chemical characteristics of the 0–40 cm soil profile detected at the beginning of the experiment (October 2019) from the average of 36 samples (average ± SE).

Soil characteristics	Values	Method
Sand, 2000–50 µm (%)	36.9 ± 5	Standard sieve-pipette method (ISO 11277, 2009)
Silt, 50–2 µm (%)	44.1 ± 5	
Clay, < 2 µm (%)	19.0 ± 2.2	
pH	8.0 ± 0.2	Dual meter
EC 1:2.5 (mS cm^{-1})	0.19 ± 0.02	pH/conductivity (soil/water solution with ration 1:2.5)
Organic carbon (%)	0.81 ± 0.1	CNS elemental analyzer
Inorganic carbon (%)	4.25 ± 0.2	
Total Kjeldahl nitrogen (%)	0.09 ± 0.01	Kjeldahl method
NO_3-N (mg kg^{-1})	56.6 ± 18.1	Ion Chromatography (after water extraction)

2.2. Experimental layout

The research was conducted for 2 consecutive growing seasons (2019–2020 and 2020–2021) adopting an experimental layout with 3 CCs treatments x 2 replicates x 2 blocks. The experimental site consisted of a 5.5 ha area composed of 12 plots (0.3–0.5 ha each) divided into two blocks (of 6 plots each), separated by a PVC film buried up to a depth of 1.5 m. Each block contained 2 plots (replicates) for each of the 3 CCs treatments. The study factor consisted of 3 winter CC treatments introduced in a silage maize production system. Specifically, the tested CC treatments were: (i) a fixed treatment (FI) where triticale (x *triticosecale*) was used as CC in both seasons of experimentation; (ii) a 2-year succession (SU) of rye (*Secale cereale* L. in 2019–2020) and crimson clover (*Trifolium incarnatum* L. in 2020–2021); and (iii) a weed-covered control (NoCC) where any CC was cultivated in both experimental seasons and any weeds control (mechanical or chemical) was applied.

2.3. Crop management

The experimental site had been managed conventionally since 1996 with a non-strict rotation of maize (*Zea mays* L.), wheat (*Triticum aestivum* L.), soybean (*Glycine max* (L.) Merr.), sorghum (*Sorghum bicolor* L.) and sugar beet (*Beta vulgaris* var. *saccharifera* L.) (Tolomio and Borin, 2019). The CCs were introduced in autumn 2019. Maize (Pioneer P 2088 – FAO 700) was sown with 0.75 m inter-spacing on April 17th 2020 and April 26th 2021, and harvested for silage at the end of August (August 28th 2020 and August 25th 2021). The CCs were sown on October 10th 2019 at a seeding rate of 160 kg ha^{-1} for rye and triticale, and on October 9th 2020 at seeding rates of 40 kg ha^{-1} for crimson clover and 160 kg ha^{-1} for triticale. CC termination occurred by shredding with a rotary mulcher on March 31st of both years.

Agronomic field management in the two seasons included, after CC termination, subsoil tillage (at 30 cm depth) and harrow rolling for cash crop seedbed preparation. Maize was irrigated once in each season (40 mm in 2020 and 30 mm in 2021). It was mineral fertilized in each growing season with 200 kg N ha^{-1}, 80 kg P_2O_5 ha^{-1}, and 80 kg K_2O ha^{-1}. Fertilization was carried out before sowing, except for the N that was supplied as urea partially before sowing (32 kg N ha^{-1}) and the rest as one top-dressing. Weeds were controlled with the pre-emergence application of terbuthylazine, S-metolachlor and mesotrione, as well as with post-emergence mechanical control. Lamdex® Extra-Adama (active ingredients: pure lambda-cyhalothrin 25 g kg^{-1}; application rate: 1 kg ha^{-1}) was applied post-emergence (half of July in both 2020 and 2021) for pest control.

2.4. Meteorological variables and growing degree days

Rainfall, air and soil temperature were monitored by a meteorological station (ARPAV) located within the experimental site.

2.5. Sampling

2.5.1. Crop sampling

The aerial part and the root biomass of the CCs were sampled each year at CC termination on March (March 31st 2020 and 2021). The aerial CC biomass was also sampled during both growing seasons at the end of January (January 31st 2020 and 2021) and February (February 28th 2020 and 2021). On each sampling date, the CC samples were collected in 3 georeferenced sampling points within each plot for a total of 12 samples *per* species (3 samples x 2 replicates of each CC treatment x 2 Blocks). The aboveground CC biomass was collected manually from 4-m^2 sample areas and it was visually inspected to identify the main species composition, while the roots were sampled from a 0.5 * 0.5 * 0.5-m cube of soil (each year). They were separated from the soil by applying the wet hand washing method described by Smit et al. (2013). Maize was monitored for its growing status 39 and 73 days after sowing in 2020 and 2021, respectively. Plant height (m) and SPAD measurements were performed on 20 plants in three points of each experimental plot. Maize (whole plant) aboveground biomass was sampled at the silage stage in each plot from 3 areas of 18 m^2 consisting of 2 rows of 12 m length x 0.75 m inter-row. The CC and maize dry matter contents were determined by drying the biomass in a thermo-ventilated oven at 65 °C. All the dried biomasses were then chopped and analyzed for their N and carbon (C) contents using a CNS Vario Macro elemental analyzer (Elementar, Hanau, Germany). N uptake and biomass fixed C were determined by multiplying their concentration in the dry biomass produced *per* unit area.

2.5.2. Soil sampling

Soil samples (3 in each plot at 0–40 cm depth) were collected in 2019, at the beginning of the experiment, right before CC sowing, and then each year at the termination of each cover crop and at the harvesting of cash crops season, respectively. All the samples were collected in 3 georeferenced sampling points (the same as those used for CC biomass) for each main plot. The soil samples were collected with an auger and left to air-dry outdoors. The dried samples were sifted using a 2-mm sieve and stored in falcon tubes before being analyzed for their nitrate (NO_3^-) content (Nmin).

2.6. N dynamics

2.6.1. Maize N use efficiency

NUE was calculated at the end of each year, together with an output-to-input ratio where (i) fertilizer N, soil Nmin content at maize sowing, and the total aboveground and root N content (kg ha^{-1}) of the CC biomass were considered as N inputs, and (ii) N uptake (kg ha^{-1}) by maize at the silage stage was included as an output.

The desirable range for the NUE area was built by applying graphical NUE representations, as suggested by the EU Nitrogen Expert Panel (EUNEP, 2015) and reported by Quemada et al. (2020). The Euclidean space was built using a line for the accepted minimum N uptake (80 kg ha^{-1} $year^{-1}$), the use efficiency (UE) = 0.50, the desired maximum surplus to avoid substantial pollution by N losses (UE = 0.80) and the UE = 0.90 lines (as reported in the EUNEP, 2015). The desired minimum N uptake line represents the lower limit to obtain acceptable crop production, while the UE = 0.50 and UE = 0.90 lines represent the lower and upper boundary efficiencies to minimize nutrient loss into the environment and soil mining, respectively. Finally, the desired maximum surplus line delimited the maximum acceptable difference between the input and the output.

2.6.2. Apparent mineralization and immobilization index

An apparent N mineralization-immobilization index (ANMI; kg ha^{-1}) was calculated to quantify the apparent quantity of N mineralized or immobilized during the maize season after the incorporation of different CC residues. The index was based on the previous "apparent N mineralization" (ANM) formula reported by Hartmann et al. (2014). The ANMI was calculated assuming that gaseous N emissions equalled atmospheric N depositions, and N leaching was negligible (water drainage was never observed from the site during the experimental period).

The ANMI was computed for each year using the following formula:

$$ANMI = [\text{Maize N uptake} + \text{Soil Nmin}_{harvest}] - [\text{Tot CC N uptake} + \text{Nfertilizer} + \text{Soil Nmin}_{sowing}],$$

where Tot CC N uptake is the amount of N uptake (kg ha^{-1}) of the CC aboveground and root biomasses; Nfertilizer is the quantity of N applied through mineral fertilization; Soil $Nmin_{sowing/harvest}$ is the soil NO_3^- content at the maize sowing (before N fertilization) and harvest times; and Maize N uptake is the N uptake by maize at harvest time.

2.7. Statistical analysis

Statistical analyses were performed using linear mixed models ('lmer()' function in R software; Bates et al., 2015) including the CC treatments, the sampling date and their interaction within each year as fixed factors, and the block as a random effect, to analyse the CCs production quantity and quality, as well as the soil Nmin content during the winter season Moreover, all the parameters were compared in 2020 *vs.* 2021 using a linear mixed model with the year and the block as fixed and random factors, respectively. Another statistical analysis was performed to investigate the effect of the CCs on the cash crop and the N dynamics. Specific mixed models for repeated measurements were used to investigate the effect of the CCs on the maize growth indices and yield production and quality over the two years of experimentation, as well as on the soil Nmin content, NUE and the ANMI. The mixed models were built including the CC treatment, the year, and their interaction as fixed effects, whereas the block and the year (repeated measurements) were included as random factors in a nested structure, as reported by Onofri et al. (2016).

Marginal and conditional residual distributions were checked visually to detect possible issues of non-normality or heterogeneity of variances. A Wald test ANOVA of each model was performed to confirm the results of the models, and *post-hoc* analyses were carried out using the emmeans package in R with Sidak's test for multiple sets of pairwise comparisons or Tukey's test for one set of pairwise comparisons (Lenth et al., 2021). All the statistical analyses were performed using R software (R Core Team, 2021).

3. Results

3.1. Meteorological data

The cumulative precipitations recorded during the experiment (Fig. 1) were 16.6% and 21.5% lower than the 25-year average (841 mm) for 2020 and 2021, respectively. During the CC seasons (October-March), the distribution of rainfall differed between the two years: cumulative values were 380 mm in 2019–2020, and 279 mm in 2020–2021 (25-year average: 384 mm). In the first 3 months after CC sowing, the 2 seasons differed, in particular for precipitations in November (150 mm in 2019 *vs.* 14 mm in 2020), whereas the distribution was opposite in the following 3 months. March was the rainiest month in 2019–2020 (60 mm), while the highest precipitation was measured in January of the following autumn-spring season (72 mm), followed by a decrease in the next 2 months. Soil temperature reached the average maximum value equal to 25 °C in July and a minimum temperature of 4.8 °C in January.

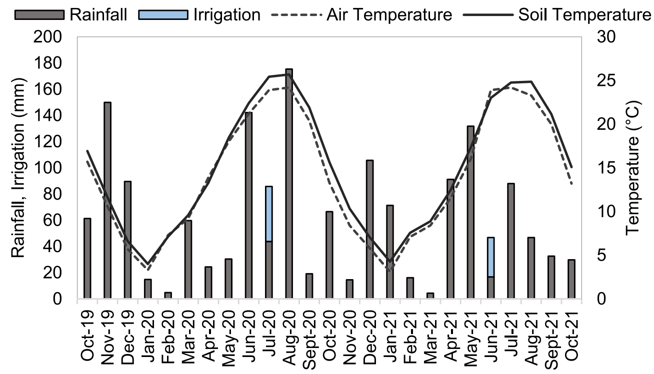


Fig. 1. Monthly mean temperature (air temperature and soil temperature at 20 cm depth) and cumulative rainfall, and irrigation events during the 2-year experimental period in Legnaro (Padova, Italy).

3.2. Winter cover crops and soil Nmin

The CC aboveground dry biomass (Fig. 2) was significantly affected by the CC type in interaction with the sampling date over the two years. In 2020, both grasses had the same quantity at the end of January; in February, triticale accumulated more dry biomass (+ 77.1%) than rye (1.1 Mg ha^{-1}) keeping it constant until the end of March, when instead the rye showed the highest biomass production (2.5 Mg ha^{-1}; Fig. 2). Weeds developed in the NoCC treatment and produced almost steady biomass throughout the winter season (0.4 Mg ha^{-1} on average), much lower than those of rye and triticale at each sampling date.

In 2021, despite the CC treatments, the highest biomass quantity was measured at termination time, including NoCC treatment (1.9 Mg ha^{-1} on average). Triticale showed the same biomass production from the end of January until termination time. Clover and NoCC experienced slower growth than triticale until the end of January but eventually achieved the same yield as triticale at termination time (Fig. 2).

Comparing the treatments in 2020 *vs.* 2021 the main difference was observed in the NoCC where weed biomass in 2020 was 80.1% and 69.1% lower at the end of February and March than at the same sampling dates in 2021 (1.5 Mg ha^{-1} and 2.1 Mg ha^{-1}, respectively).

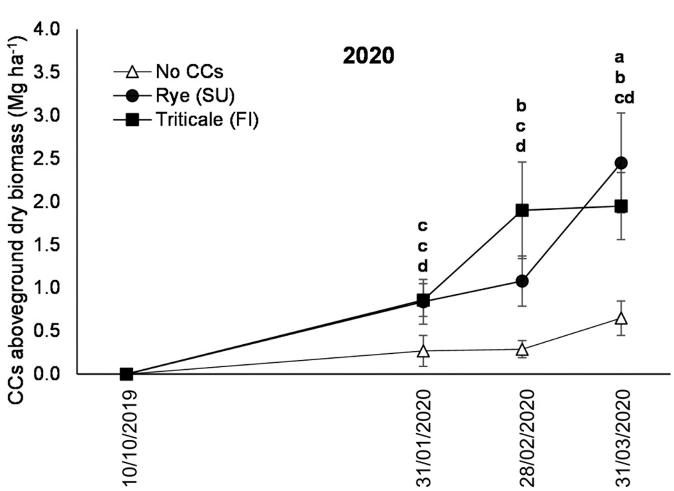
The N uptake by the CC aboveground biomass (Fig. 3) was significantly affected by the CC treatment in interaction with the sampling date in 2020 and 2021. In the first year, rye displayed the highest N uptake between treatments at termination time. A significantly lower N uptake (−33.3%) was measured for triticale. Weeds showed the lowest N uptake compared with all the CC treatments over the entire 2020 season. Both CC species presented a lower (−84.1%) N uptake in 2020 than in 2021, following the biomass trend. In 2021, the highest N uptake was measured for weeds and clover at termination time (52 kg ha^{-1} on average), whereas triticale showed a lower N uptake (−35.2%).

The CC type significantly affected root dry biomass production and N uptake at termination time in both years (Table 2). In 2020, triticale and rye produced the same biomass (4.8 Mg ha^{-1} on average), 8.4 times more than weeds. The same result was observed for N uptake, which was the same for both grass species (50.5 kg ha^{-1} on average) and higher than that of weeds (7.4 kg ha^{-1}). In 2021, the weed root biomass was similar to that of triticale (2.8 Mg ha^{-1} on average) and higher than that of clover (1.9 Mg ha^{-1}). However, the same root N uptake was measured in all treatments (23.4 kg ha^{-1} on average).

Root biomass of the NoCC (2.1 Mg ha^{-1}) and SU (1.9 Mg ha^{-1}) treatments were 3.2 times higher and 6.3 times lower in 2021 than in 2020, respectively. Similarly, the root N uptake of the NoCC treatment was 1.7 times lower in 2020 than in 2021 (20.2 kg ha^{-1}), whereas the root N uptake of the SU treatment was 1.2 times higher in 2020 than in 2021.

The C:N ratio of the aboveground biomass (Table 3) was significantly higher in the FI treatment (+37.5%) than in the NoCC treatment (24) in 2020, while rye was in between. In 2021, this same ratio was 46.6% higher in the FI treatment than in the NoCC and SU treatments (15). The C:N ratio of the root biomass (Table 3) was higher in the FI and SU treatments (+33.3%) than in the NoCC treatment (21) in 2020, where spontaneous clover was observed through a visual inspection. In 2021, the C:N ratio of the FI treatment (38) was higher than that of the SU treatment (25), while clover was in between.

The sampling date and the interaction between the CC treatment and the sampling date significantly influenced the soil Nmin content in both years (Fig. 4). In 2020, the highest contents were recorded under all treatments at the beginning of the experiment (October 2019) (82.9 kg NO_3^--N ha^{-1} on average). During the winter season, the Nmin content decreased in all conditions, but was higher in the NoCC treatment (43.1 kg NO_3^--N ha^{-1} on average) compared to SU (−55.8%) in January and both FI and SU (−53.4% on average) on February. At the end of March, no difference in Nmin content was measured among the three CC treatments (29.1 kg NO_3^--N ha^{-1} on average). In 2021, the highest Nmin values were measured at CC sowing (37.7 kg NO_3^--N ha^{-1}), whereas the lowest (16.9 kg NO_3^--N ha^{-1}) at the end of February with January and March showing any significant difference. Soil Nmin content at the end of January and March instead didn't show any significant difference (30.6 kg NO_3^--N ha^{-1} on average).



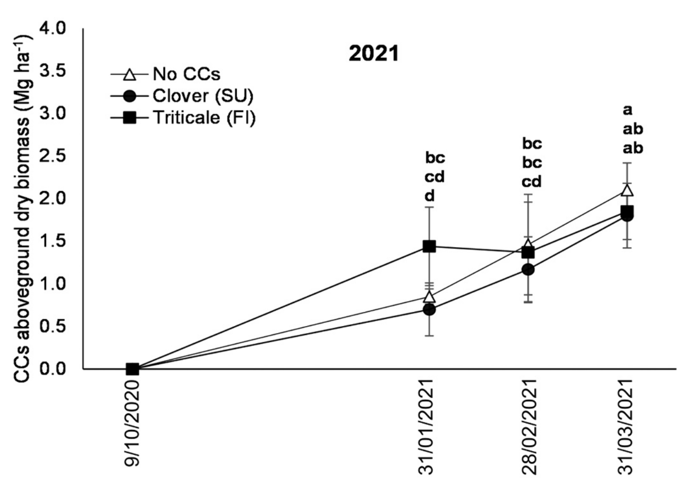


Fig. 2. Cover crops (CCs) aboveground dry biomass (Mg ha^{-1}) in 2020 and 2021 (average ± SE with 0.95 confidence interval, reported with the vertical bars). Different lowercase letters indicate significant differences among treatments and sampling dates within each year. Significance (p value ≤ 0.01) obtained with Sidak post hoc test. No CCs= absence of cover crops; SU= succession treatment; FI= fixed treatment.

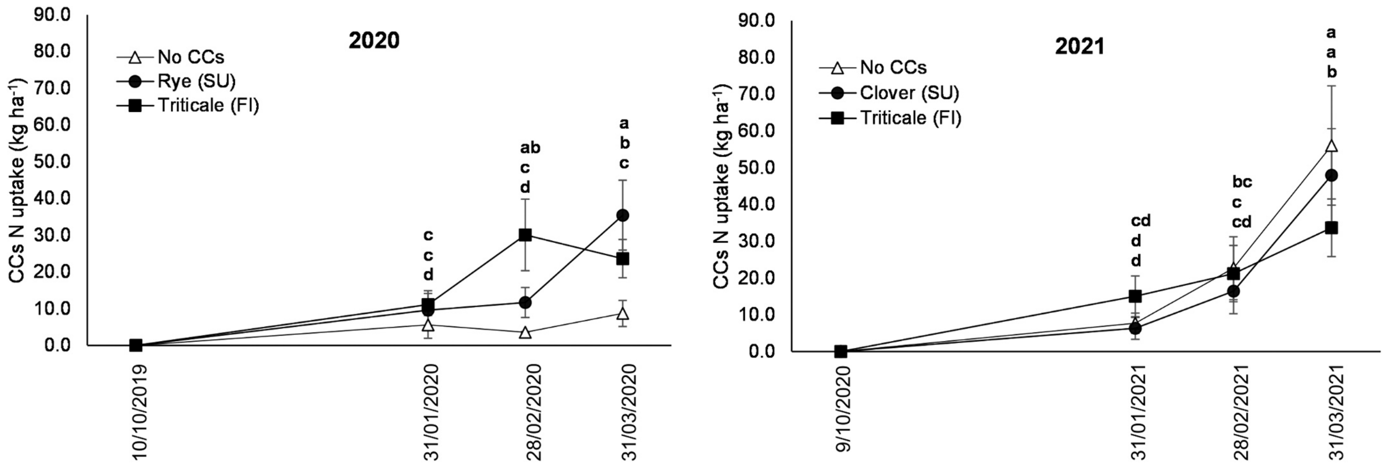


Fig. 3. Cover crops (CCs) aboveground dry biomass N uptake (kg ha^{-1}) in 2020 and 2021 (average ± SE with 0.95 confidence interval, reported with the vertical bars). Different lowercase letters indicate significant differences among treatments and sampling date within each year. Significance (p value ≤ 0.01) obtained with Sidak post hoc test. No CCs= absence of cover crops; SU= succession treatment; FI= fixed treatment.

Table 2
Cover crops (CCs) root dry biomass (Mg ha^{-1}) and N uptake (kg ha^{-1}) in 2020 and 2021 (average ± SE with 0.95 confidence interval). Different letters indicate significant differences within each year. Significance (p value ≤ 0.01) obtained with Sidak post hoc test. ns = not significant differences.

Year	CCs Treatments	Roots Biomass (Mg ha^{-1})		Roots N uptake (kg ha^{-1})	
2020	No CCs	0.5 ± 0.05	b	7.4 ± 5.3	b
	Rye	5.1 ± 2.4	a	52.5 ± 20.8	a
	Triticale	4.7 ± 1.1	a	47.6 ± 9.4	a
2021	No CCs	2.1 ± 1.1	a	20.2 ± 9.2	ns
	Clover	1.9 ± 0.9	b	24.6 ± 9.2	ns
	Triticale	3.5 ± 0.7	a	25.7 ± 5.2	ns

Table 3
Cover crops (CCs) aboveground and roots C:N ratio in 2020 and 2021 (average ± SE with 0.95 confidence interval). Different letters indicate significant differences at $p < 0.001$.

Year	Treatment	C:N ratio			
		Aboveground biomass		Roots biomass	
2020	No CCs	24 ± 5.1	b	21 ± 4.4	b
	Rye	27 ± 3.2	ab	29 ± 3.2	a
	Triticale	33 ± 2.1	a	27 ± 2.7	a
2021	No CCs	15 ± 0.9	b	33 ± 2.3	ab
	Clover	15 ± 1.3	b	25 ± 1.1	b
	Triticale	22± 1.7	a	38 ± 1.7	a

3.3. Maize biomass, N uptake, and soil N min

3.3.1. Maize growth monitoring

Maize growth was monitored 39 and 73 days after sowing in 2020 and 2021, respectively. The SPAD values of the 2 CC treatments significantly differed from those of the NoCC treatment (Table 4). In 2020, the SPAD values of maize grown after each CC were significantly lower (24.6 on average) than those of maize following weed-covered control treatment (34.9). In 2021, only the SPAD values of maize grown after the FI treatment were significantly lower (30.1) than those of maize grown after the NoCC and SU treatments (40.4 on average). No significant difference in maize height was observed in 2021 *vs.* 2020.

3.3.2. Maize yield and soil Nmin

The CCs did not affect the yield of the following maize crop (Table 5). However, the maize dry biomass yield was significantly higher in 2020 (17.9 Mg ha^{-1}) than in 2021 (15.4 Mg ha^{-1}). Differently, the CCs treatment affected the maize N uptake, which resulted significantly higher in the NoCC than both the CCs treatments in 2020 (+ 40% than 140.5 kg ha^{-1} on average) and only triticale in 2021 (+16% than 178.6 kg ha^{-1}).

Soil Nmin content at maize sowing was affected by the treatments in interaction with time, showing significantly lower values in the triticale treatment of both years (21.1 kg NO_3^--N ha^{-1} on average) compared to NoCC of 2020 and 2021 and clover 2021 (35.5 kg NO_3^--N ha^{-1} on average), with rye showing any significant difference. A similar result was observed for the residual soil Nmin content at harvest in 2020 (where the FI treatment resulted in the lowest values), whereas no difference was observed among treatments in 2021.

3.4. N dynamics

3.4.1. Maize N use efficiency and apparent N mineralization and immobilization index

The NUE of maize in the two years is represented graphically in Fig. 5, while its mean values are reported in Table 6. The CCs differently affected NUE. The highest and lowest maize NUE values were measured in 2020 following the NoCC (77.7%) and rye (46.2%), respectively; intermediate values were obtained in other treatments (Fig. 5). NoCC in 2020 was the only treatment that led to a distribution of the values close to the desirable NUE range. In 2021, the distribution of all NUE values fell within the graphical space between the desirable range and the 50% threshold. Compared to 2020, in 2021 the NUE of maize decreased in the NoCC treatment, while it increased in the SU treatment.

The ANMI (Table 6) showed a similar pattern to that of the average NUE values except for maize cropped after triticale in 2020. The ANMI was significantly influenced by the treatment in interaction with the years: the highest value (23.2 kg ha^{-1}) was measured following the NoCC treatment of 2020, and the lowest values following both CC treatments with grass species in the same year (−111.6 kg ha^{-1} and

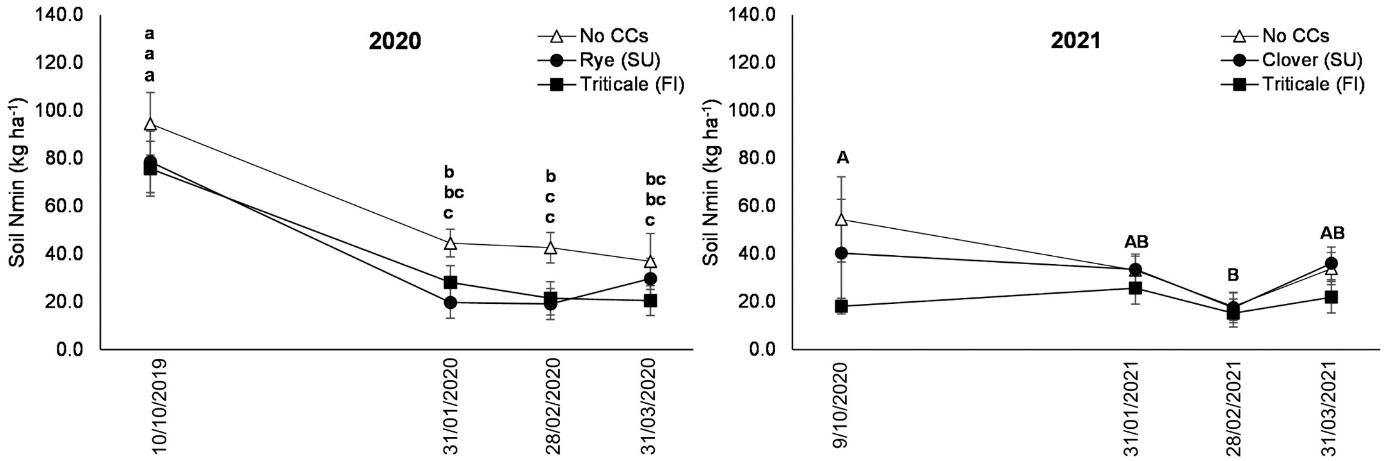


Fig. 4. Soil Nmin (NO_3^--N) (kg ha^{-1}) in the first 0–40 cm soil layer in 2020 and 2021 (average ± SE with 0.95 confidence interval, reported with the vertical bars). Different lowercase letters indicate significant differences among treatments and sampling date; different uppercase letters indicate significant differences among sampling dates. Significance (p value ≤ 0.01) obtained with Sidak post hoc test. No CCs= absence of cover crops; SU= succession treatment; FI= fixed treatment.

Table 4
Maize SPAD values and height (m) at 39 and 73 days after sowing in 2020 and 2021, respectively (average ± SE with 0.95 confidence interval). Different lowercase letters represent significant differences ($p < 0.05$) among treatments in the same year. ns = not significant differences.

Year	CCs Treatments	SPAD		Height (m)
2020	No CCs	34.9 ± 4.2	a	0.56 ± 0.09
	Rye	24.8 ± 4.6	b	0.45 ± 0.08
	Triticale	24.5 ± 4.2	b	0.37 ± 0.08
2021	No CCs	36.6 ± 5.6	a	1.8 ± 0.3
	Clover	44.3 ± 10.2	a	2.0 ± 0.3
	Triticale	30.1 ± 4.7	b	1.9 ± 0.2
Treatment		*		ns

−131.2 kg ha^{-1} for rye and triticale, respectively). The ANMI following all the CC treatments of 2021 (85.4 kg ha^{-1} on average) were significantly lower than those following the NoCC treatment in 2020 but higher than following both CC species in 2020.

4. Discussion

Winter CC aboveground and root biomass accumulation can determine the extent of several CC effects. Among these effects, there is the potential control of winter soil N losses (McGourty and Reganold, 2005), especially in the case of grass species (Chen and Weil, 2010). The present investigation of triticale and rye biomass accumulation during the winter season, along with the soil Nmin content, confirms both species as valid catch crops, consistently with previous findings (Ruffo and Bollero, 2003). Despite different growth patterns of triticale and rye, both grasses equally reduced soil Nmin compared to the NoCC treatment throughout the winter season, and no difference between the two was observed in the residual soil Nmin content at termination at the end of March, confirming the findings of Thapa et al. (2018). Moreover, no difference in maize production or N uptake was evidenced after the two grasses, which rules out a higher preemptive N competition after one of these two species. Even though triticale had accumulated a higher aboveground biomass and displayed a higher N uptake than rye by the end of February, rye outperformed triticale at termination time, which maintained the same biomass quantity produced in the previous month. In a humid-subtropical climate zone (North Carolina, USA), rye had higher biomass and N uptake than triticale only when terminated later on in the spring season (end of April/May) (Komatsuzaki and Wagger, 2015). In our study conducted in a similar climate zone, rye performed as depicted by these authors as early as at the end of March.

A similar performance in terms of biomass production and reduction of the soil Nmin content during the winter season was observed with triticale and crimson clover in the second year. Although crimson clover initially had a lower biomass and a lower N biomass content than triticale, it recovered from February to March, and even had a higher N content in its total biomass than triticale at termination. Despite similar biomass production to triticale, the aboveground biomass of clover at termination in our study was slightly lower than the average values measured in sub-humid regions (Ruis et al., 2019), and the range of biomass production (3–5 Mg ha^{-1}) reported by Lu et al. (2000). Our results disagree with previous studies reporting higher aboveground (Brennan and Smith, 2005; Kaspar and Singer, 2011) and root (Amsili and Kaye, 2021) biomass production by winter grasses compared to crimson clover. It is indeed stated that crimson clover usually starts its biomass accumulation later than grass crops do – in late spring.

The high percentage of root biomass compared with the whole biomass and N uptake of all CC species suggests that the root system played an important part in the CC-cash crop rotation system. Besides aboveground biomass, the roots might play a crucial role in determining several effects for which the CCs are usually introduced in agricultural systems (Amsili and Kaye, 2021). The root biomass has been widely related to the plant's ability to acquire, use and conserve N resources by affecting the N nutrient cycle (Reich et al., 2003; Wendling et al., 2016). Therefore, it is fundamental to include root biomass production besides aboveground biomass production for any reliable investigation on the N cycling processes, especially since literature about the root biomass is scarce, as it has not received as much attention as aboveground biomass (Roumet et al., 2006).

The aboveground and root biomass production of weeds in the NoCC treatment highly increased as early as the second year of experimentation. This suggests that an agricultural field left as fallow and without any weed control (chemical or mechanical) over winter can significantly increment the presence of weeds (in a different measure according to the seasonality and tillage system) in the short term, with the risk of increasing the winter weed seed bank. In 2021 indeed, the weed biomass reached the same level as those of the other CCs, with even higher N uptake at termination. Moreover, the weed biomass quality (C:N) was similar to that of clover (possibly related to the presence of spontaneous leguminous species and very young spontaneous vegetation with low lignin content) suggesting potential similar residue decomposition and mineralization after incorporation. Further research should be conducted in this direction to investigate the potential – positive and negative – effects of spontaneous vegetation growing in the fallow period.

Table 5
Soil Nmin content (kg ha^{-1}) at maize sowing and harvest time; maize biomass yield (Mg ha^{-1}), and N uptake (kg ha^{-1}) at harvest time (average ± SE with 0.95 confidence interval). Significance codes: * ** =$p < 0.001$; * =$p < 0.05$; ns = not significative.

Year	CCs Treatments	Biomass yield (Mg ha^{-1})		Biomass N uptake (kg ha^{-1})		Soil Nmin at maize sowing (0-40 cm) (kg ha^{-1})		Soil Nmin at maize harvest 0-40 cm (kg ha^{-1})		
2020	No CCs	19.3 ± 2.7	A	196.7 ± 9.7	a	36.8 ± 7.1	a	79.6 ± 26.1	a	A
	Rye	17.5 ± 2.6		147.1 ± 6.6	b	29.6 ± 6.3	ab	59.1 ± 32.8	a	
	Triticale	16.9 ± 2.1		134.0 ± 9.4	b	20.4 ± 2.2	b	26.5 ± 4.7	b	
2021	No CCs	16.5 ± 2.8	B	207.4 ± 10.1	a	33.8 ± 6.8	a	17.3 ± 5.9	ns	B
	Clover	14.2 ± 4.3		187.5 ± 9.5	ab	36.1 ± 8.4	a	25.6 ± 7.1	ns	
	Triticale	15.5 ± 2.3		178.6 ± 9.8	b	21.9 ± 3.2	b	26.7 ± 8.8	ns	
Year		*		ns		ns		*		
Treatment		ns		***		***		*		
Year x Treatment		ns		ns		*		ns		

Understanding N availability at cash crop sowing is crucial information that can help landowners manage N fertilization more efficiently. N availability for the following cash crop usually depends on several factors such as the decomposition rate of the CCs (C:N ratio), NO_3^- and NH_4^+ availability in the soil, carbon availability, and the aeration status of the soil (Davidson et al., 2000; Rosecrance et al., 2000).

We used SPAD measurements in the first stages of maize development as proxies of N availability after the different CC treatments. In accordance with previous studies (Rosecrance et al., 2000; Ruffo and Bollero, 2003), the results showed lower N availability after both grass species than after clover and weed-covered control (in the second year). The lower soil Nmin content following triticale at maize sowing, along with the biomass production and quality (high C:N ratio) of the grass residues compared to clover and weeds (in the second year) probably left less N resources available for maize in the first month of growing. However, the lack of direct measurements of N mineralization and immobilization activities and N fertilization of maize prevented a specific description of the extent to which these processes can be attributed to the incorporation of CC residues. In their review, Kaspar and Singer, 2011 report that when N fertilization is applied at cash crop planting, the N coming from this source is recovered by the cash crop in a greater proportion compared to the N contained in the CC residues. Other studies using labelled N demonstrated that higher percentages of N (40%) from fertilizers are usually taken up by cash crops compared to CC residue sources, even if the percentage can vary according to the specific C:N ratio of the CC species (4% with rye; 17% with leguminous CCs) (Kaspar and Singer, 2011).

Despite the differences in SPAD values, the final yield of maize did not differ among treatments, suggesting that maize can recover after initial lower N availability. This is a crucial result because the impact of winter CCs on the final cash crop yield is one of the limiting factors that might prevent farmers from adopting CCs (Singer et al., 2007). Our results are in line with previous findings by Marcillo and Miguez (2017) showing that grass CCs do not significantly change (increase or decrease) maize yield on average compared to fallow. Rye has been reported to have a negative effect on maize yield when terminated four weeks later than early termination in spring (Krueger et al., 2011), possibly due to higher N immobilization after termination (Hunter et al., 2021), and the resulting delay in maize planting. CC termination in early spring (at the end of March in our site) likely prevented maize yield depletion after the rye crop, leaving time for residue decomposition (Hashemi et al., 2013), as well as reducing the potential allelopathic effects of this species (Kelton et al., 2012). The similar maize yields after the clover, triticale, and NoCC treatments in 2021 might be related to the N fertilization applied to the maize crop, as demonstrated in previous studies (Miguez and Bollero, 2006; Marcillo and Miguez, 2017). N applied at 200 kg ha^{-1}, as in our experiment, may indeed inhibit the ability of leguminous crops to increase maize yield. Clover did not increase the final N uptake by maize compared to the weed-covered control treatment, contrary to the results of Maltas et al. (2009), Gabriel and Quemada (2011), and Salmerón et al. (2011). However, this result should be evaluated considering that the biomass quantity and quality (C:N ratio) of the weeds was the same as that of the clover CC due to the presence of spontaneous leguminous species and young vegetation in the experimental site. The lower final N uptake by maize after the grass crops confirms the findings of Kaye et al. (2019) and suggests apparent N immobilization fostered by the incorporation of grass residues (high C:N ratio). This observation is strengthened by the computation of the ANMI. The ANMI showed a residual Nmin quantity at the end of each CC-maize cycle in 2020 and 2021. This quantity was significantly higher after both grass CCs in 2020 compared to all other treatments, suggesting apparent N immobilization after the incorporation of grass residues in 2020. However, in the absence of specific measurements of the mineralization and immobilization processes, this process can be likely also attributed to other factors such as microbial N

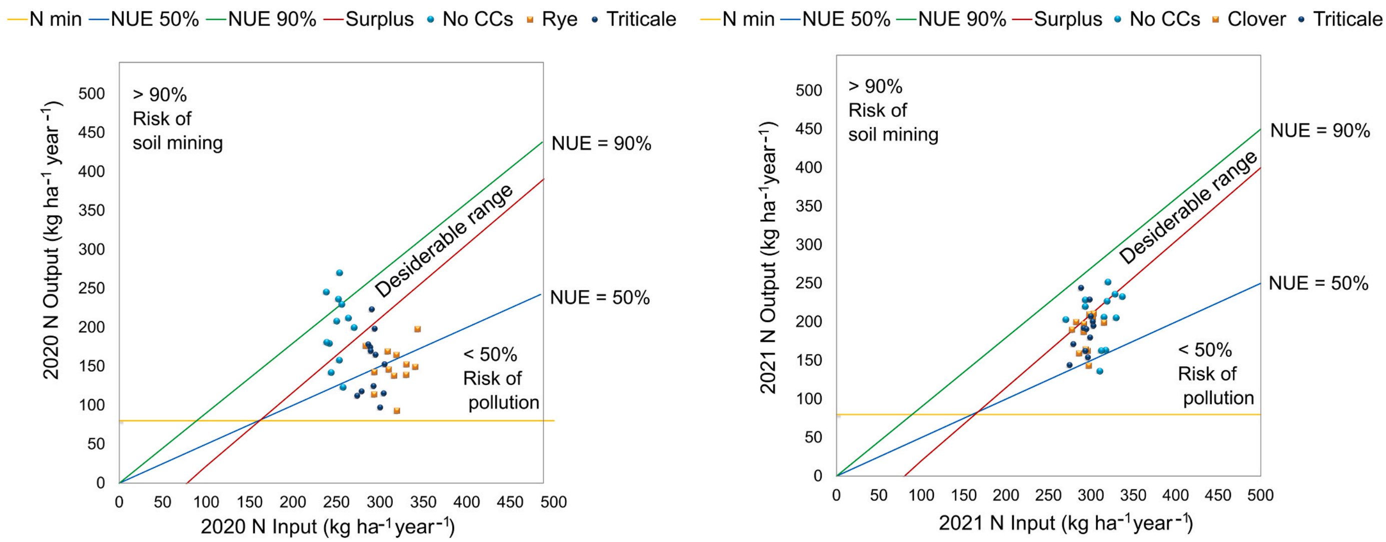


Fig. 5. Graphical presentation of the N use efficiency (NUE) (according to the EU Nitrogen Expert Panel, 2015) in both 2020 and 2021 for each CCs and fallow treatments. The green line corresponds to the NUE = 90%; the red line to the desired maximum surplus (NEU = 80%); the blue line to the NUE = 50%; the yellow line to the desired minimum productivity. The "Desiderable range for NUE" is the area ranging from the green and the red lines.

Table 6
Average N use efficiency (NUE) (%) and apparent mineralization-immobilization index (ANMI Index) (kg ha^{-1}) for each CCs treatment in 2020 and 2021. Significance codes: * ** =p < 0.001; * * = p < 0.01; ns = not significative.

Year	CCs Treatments	NUE (%)		ANMI Index (kg ha^{-1})	
2020	No CCs	77.7	a	23.2	a
	Rye	46.2	c	-111.6	c
	Triticale	52.3	bc	-131.2	c
2021	No CCs	66.8	b	-85.4	b
	Clover	60.7	b	-95.4	b
	Triticale	63.5	b	-75.5	b
Year		ns		ns	
Treatment		***		***	
Year*Treatment		**		**	

immobilization (for their activity or their constitution) (Lima et al., 2022) and N immobilization in the maize residues left in the field after harvest (especially the roots) (Torma et al., 2018). NoCC in the first year was the only treatment that did not show any apparent immobilization but rather apparent mineralization. This observation can be strengthened by looking at the distribution of NUE data in this treatment, which was the only one within the desirable range areas of the EU Nitrogen Expert Panel (EUNEP, 2015). For the grass CC species, the distribution of NUE values in 2020 was around 50% of the EUNEP. This result suggests a potential risk of N leaching and/or immobilization (as no significant leaching was observed during the experiment). In 2021, all treatments showed higher NUE than triticale and rye in 2020. Therefore, different CC species and years, as well as the same species in different years (triticale in our study), might differently stimulate N mineralization-immobilization processes, as already reported (Thapa et al., 2021). However, many other factors such as the soil microbial activity, drying and rewetting events, the soil characteristics, and the interaction among all these variables (Cabrera et al., 2005) may have affected the conversion of organic N into ammonium N, or of inorganic N into an organic form. Analyzing all these factors and their interaction is crucial to understand N cycling in soils and efficiently use CC organic residues as an available source of N for subsequent cash crops. For this reason, further analyses will be conducted to study in depth the N mineralization-immobilization processes following CC residue incorporation.

5. Conclusions

The present study shows that maize yield was not affected by the introduction of winter CCs. However, CCs impacted the N dynamics. High apparent N immobilization as well as reduced N uptake and NUE by maize were measured after rye and triticale winter CCs compared to clover and NoCC. Nevertheless, both grass species reduced the soil Nmin over the winter season, acting as valid catch crops. Triticale developed faster than rye and crimson clover. However, this latter at termination produced the same biomass quantity as triticale did, but with a higher N content and a lower C:N ratio determining lower apparent N immobilization during the following maize growing season. This suggests that clover might be an appropriate option for the first year of CC introduction to prevent potential N resource immobilization related to the incorporation of grass CC residues.

Besides evaluating the impact of CCs on cash crop yield, understanding their impact on N cycling is necessary to optimize their use and select the best possible CC species and management options. Therefore, it will be fundamental for future perspectives to use appropriate instruments to accurately measure the N inputs of the CCs and the meteorological variables that can affect the soil N mineralization-immobilization processes related to the decomposition of CC residues, and refine the N fertilization balance of the cash crop accordingly. Indeed, these processes determine N availability during the succeeding cash crops season and affect N uptake by maize, even when the yield is not affected by CC introduction. Lastly, the results suggest that CCs research should include root biomass production, which can represent a high percentage of the total biomass and many times is not considered.

CRediT authorship contribution statement

Giorgia Raimondi: Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing. **Carmelo Maucieri**: Conceptualization, Methodology, Investigation, Data curation, Writing – original draft, Writing – review & editing, Supervision. **Maurizio Borin**: Conceptualization, Methodology, Writing – review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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