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CROP RESPONSE, N AND C DYNAMICS AFTER COVER CROP INTRODUCTION

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Abstract

Cover crops (CCs) exert varying influences on the soil nitrogen (N) cycle and water content (SWC) throughout different crop rotation seasons. A thorough assessment of CC growth patterns, nitrogen accumulation, and mineralization holds crucial importance in optimizing their effects on N, SWC resources, and cash crop performances. Furthermore, introducing CCs into cropping systems alongside organic fertilizers is regarded as a pivotal strategy for enhancing short-term soil organic C (SOC) levels.

Within this context, the present thesis examines the short-term effects of introducing CCs in two northeast Italy experimental fields, aiming to achieve four objectives. The first three objectives involve studying the impact of two CC successions (in the experimental farm of the University of Padova “L. Toniolo” in Legnaro) - grasses followed by grasses; and grasses followed by leguminous and brassica species - within a 3-year maize-soybean succession experiment. Specifically, the objectives aim to: (i) evaluate CC performance (aboveground and roots production and N uptake) and their influence on silage maize production and soil nitrates (NO_3^-) content; (ii) use satellite data and model to monitor CC growth and predict their N contribution to subsequent crops; (iii) study CC effects on soil NO_3^-, N functional genes (NFGs), and their relation to SWC and crop yield across different season. The fourth objective examines the short-term impact of two CC successions (in the demo farm “Podere Fiorentina” of the local Land Reclamation Authority in San Dona di Piave) - grasses followed by grasses, brassica followed by grasses - alongside organic fertilization matrices and irrigation, within a 4-year maize-soybean crop succession in on-farm experimentation, aiming to assess their combined influence on the short-term SOC stock.

All CC treatments showed comparable yields of maize and soybean compared to fallow control (without any weeds control). However, diverse CCs exhibited distinct growth patterns and differently affected soil NO_3^- and NFGs throughout different phases of crop rotation. Satellite imagery analysis indicated that rye and triticale exhibited accelerated growth rates during the winter season compared to clover, but slower than mustard, which suffered a frost winterkilling. During the growing season both grasses CC reduced soil NO_3^- content, acting as catch crops, and potentially enhanced microbial-mediated N fixation. Conversely, clover CC exhibited greater residual soil NO_3^- compared to grasses and promoted microbial-mediated N nitrification.

Following the CC residues incorporation, the CC-NCALC model estimated a net N mineralization for all CC residues, excluding N immobilization following triticale root residues. Throughout the ensuing cash crop season, the estimated N release from clover and mustard residues was around 33%, whereas, for triticale, it was about 3% of their total N uptake, with a releasing peak 2 months after their termination. At cash crop harvest time, greater NFG abundance was measured when cultivating soybeans instead of maize. This underscored the role of cash crop species in shaping N transformation dynamics. While CCs influenced the temporal variation of SWC, none of the tested CCs competed with subsequent cash crops for water resources. Additionally, the introduction of CCs in a conventional maize-soybean succession did not notably affect short-term SOC content.

The use of remote sensing imagery and prediction models for CC residue decomposition exhibits promising potential as tools for optimizing CC utilization. Nonetheless, further analysis incorporating various CC species (including the assessment of both aboveground and roots biomass), environmental variables, and diverse cropping systems is necessary to ascertain their applicability and reliability. NFGs were sensitive biochemical N cycle indicators, but their susceptibility to various factors demands careful sampling time to distinguish the main effects under analysis while ensuring the accuracy and reproducibility of the assessment.

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Introduction

1. Cover crops overview

Agriculture stands as a pivotal sector significantly contributing to the global economy while intricately governing ecosystem services. In the 21st century, agriculture encounters many challenges that necessitate attention such as the sustenance or increase of agricultural productivity, healthy food production, the mitigation of adverse environmental repercussions of agricultural activities, the adaptation to climate change, and the assurance of economic sustainability of agricultural farms (Komarek et al., 2020). Up to the present time, to amplify the production of food, feed, and fiber, contemporary agricultural methodologies have heavily relied upon recurrent plowing, synthetic fertilizers, reactive chemicals, excessive irrigation, and mono-cropping practices (Islam et al., 2021). As a consequence, approximately 33% of global land surfaces have experienced various forms of degradation—physical, chemical, biological, or ecological (Lal, 2015) while witnessing a decline in soil ecosystem services by as much as 60% between 1950 and 2010 (Leon et al., 2014). The increasing rate of degradation ($50–100 \cdot 10^5$ ha of degraded land year^{-1}) (Vasu et al., 2020) has been worsened in recent decades by the increase in extreme events (heat waves, storms, droughts, and flooding) further exacerbating problems such as soil compaction, salinization, and erosion (Kwiatkowski et al., 2023). It is projected that approximately 20% of agricultural lands will undergo desertification by the year 2050 (Zwane, 2019). These cumulative effects render the lands unsuitable for sustained crop productivity over the long term (Kwiatkowski et al., 2023). Meanwhile, there is a global demand for an increase in agricultural production to sustain a growing population projected to reach 9.6 billion by 2050 (FAO, 2010; Zwane, 2019). In response to this increasing demand, farmers face an elevated risk of intensifying their dependence on reactive chemicals, fresh water, and energy resources (Delgado et al., 2011; Islam et al., 2021), consequently entering a detrimental cycle of unsustainability marked by the depletion and degradation of land and water resources (Scholberg et al., 2010). Within this context, it is necessary that agriculture optimize the utilization of natural and renewable resources to ensure global food security (van der Ploeg, 2021) developing agroecological solutions that enhance system resilience and better adapt to predicted climate change conditions (Challinor et al., 2014). It is noteworthy that agricultural systems in Mediterranean regions are particularly vulnerable to the impacts of climate change (Giorgi and Lionello, 2008), underscoring the crucial need for assessing various agro-environmental techniques within this context. Sustaining the health and productivity of soil is the cornerstone of sustainable agriculture, which is based on the integration of innovative and comprehensive approaches such as conservation tillage, diversified cropping techniques, cover crops (CCs), and precision soil and plant amendments. The objective is to ensure economic viability, environmental compatibility, and societal acceptability while ensuring agroecosystem services.

Within this framework, the utilization of CCs has garnered growing attention. The CCs have been indeed recently identified as a sustainable strategy for climate change mitigation and adaptation (Kaye and Quemada, 2017). They are secondary crops established not for marketable production but for various environmental benefits aimed at enhancing system sustainability (Islam and Sherman, 2021). References have documented their use for millennia. Evidence of their utilization has been reported for Ancient Chinese agriculture (Bray, 1984) or in the Mediterranean region during the Roman Empire era (Winiwarter, 2014). A successful example of their utilization throughout history is the "Norfolk rotation" in England. Around 1730 indeed it was introduced a four-year rotation of wheat (*Triticum aestivum* L.) -turnip (*Brassica rapa* L.) - barley (*Hordeum vulgare* L.) - red clover (*Trifolium pratense* L.) (Reeves, 2018) which increased wheat yields from 0.54 Mg ha^{-1} to 1.35 Mg ha^{-1} by the early 19th century. The CCs utilization in various cropping systems declined during World War II with the introduction of synthetic fertilizers (Drinkwater and Snapp, 2007a; Groff, 2015), but started to show a revival around the 1980s in South America (Brazil) (Calegari, 2003). They have been denoted by diverse terminologies across historical contexts, contingent upon the specific functions for which they have been chosen:

- When intended to absorb elements from the soil and incorporate them into their vegetative biomass they have been usually referred to as '*catch crop*' (Monteiro and Lopes, 2007; García-Díaz et al., 2017). They have been reported to effectively reduce the leaching of residual nutrients remaining in the soil after the previous crop harvest (Gabriel et al., 2013) thereby mitigating potential adverse effects on the system (Rustad et al., 2008).
- CCs have been also termed '*green manure*' for their ability to incorporate nutrients and organic matter (OM) into the soil (Quemada and Cabrera, 1997). A common example is the use of legumes to fix atmospheric N_2 for subsequent crop utilization.

However, their definition of 'catch crop' or 'green manure' might be limiting as the CCs can concurrently serve multiple functions. They have been also classified based on their temporal or permanent nature:

- Temporary covers refer to crops introduced in annual rotations, established for a period of the year, and occupying land later to be used by the cash crop.
- Conversely, permanent covers remain in place for several years and may temporally overlap with the cash crop.

Today with the increasing concerns related to environmental quality, land degradation, and global warming, there is a renewed interest in this practice (Shennan, 2008). The use of

CCs is once more becoming the pivotal element within sustainable agroecosystems aligning with recent inclinations toward green technologies and/or ecological-based and natural production systems (Baligar and Fageria, 2007).

Below are summarized the benefits and services offered by CCs, alongside their management challenges and limitations.

2. Main beneficial effects of cover crops

2.1 CC effects on soil physical properties

Enhancement of soil structure or aggregation through the influence of living and decomposing CCs material has been extensively documented. Haruna et al. (2020) reviewed several studies highlighting that the presence of CCs contributes to the augmentation of soil aggregate stability through various mechanisms, including shielding the soil surface from the impact of raindrops, the activity of the roots, supplying additional biomass, and elevating the concentrations of soil organic carbon (SOC).

The activity of the CCs roots has been reported as highly influential in terms of enhancement of soil macropores, especially when compared to fallow soil (Abdollahi and Munkholm, 2014). Plant root exudates released into the soil surrounding the roots contribute to soil particle aggregation and binding (Kirkegaard et al., 2008). In addition, thanks to the mechanism called '*bio-drilling*' the residual root channels generated by decaying roots establish pathways of least resistance within the soil profile (Kautz et al., 2013). These biopores facilitate subsequent crop root access to subsoil resources (Kautz et al., 2013). Plant species with taproots, such as the radish (*Raphanus sativus* L.) (Burr-Hersey et al., 2017), are better equipped to penetrate compacted soil layers, as their large-diameter roots are less prone to buckling or deflection, creating biopores larger than 2 mm in diameter (Han et al., 2015). Studies indicate that CCs with a fibrous root architecture, such as red fescue and oats (*Avena sativa* L.), can also penetrate compacted soil layers and induce alterations in soil porosity (Burr-hersey et al., 2017; Carof et al., 2007). Fibrous roots, characterized by a higher root length density and surface area compared to taproots, possess finer and more numerous roots. This finer root system, encountering compacted soil, can exploit smaller pores and, with a greater number of roots, exhibit heightened exploratory potential compared to taproots (Clark et al., 2003). Hence, fibrous roots can penetrate compacted soil layers, and create additional biopores (Burr-hersey et al., 2017). In addition, residues from CCs have been widely reported as able to generate temporary, transient, and permanent organic binding agents that facilitate the formation of soil aggregates (Blanco-Canqui et al. 2013; Blanco and Lal, 2023). Water-stable aggregates within CC environments are indeed reported to range from 1.2 to 2 times larger than those observed in soils without CCs (García-González et al., 2018). Additionally, even within conventional tillage practices, the presence of CCs was observed to contribute to an increase in soil aggregate stability (Qi et al., 2022). Examining different CCs (oat, blue lupin - *Lupinus angustifolius*, radish, hairy vetch - *Vicia villosa* Roth, and wheat), Reicosky et al. (2021) noted that, regardless of the species, elevated aggregate size classes, mean weight diameter, geometric mean diameter, and stability index were registered in comparison to fallow treatments. In addition, Abdollahi and Munkholm (2014) after five years of fodder radish cultivation as CC (in soil with 9% clay, 13% silt, 75% sand, and 3.1% organic matter) observed a lower penetrative resistance in the area with CC (1.62 Mpa) compared to areas without CC (1.85 Mpa) at depths ranging between 0.32 and 0.38 m.

Soil hydraulic properties. The introduction of CCs in cropping systems has been also widely reported to enhance various soil hydraulic properties including water infiltration, water retention capacity, and saturated hydraulic conductivity (Blanco-Canqui et al., 2015). By leaving open root channels, promoting increased microporosity and pore connectivity the CCs can indeed improve water infiltration within the soil (Villarreal et al., 2021), and facilitate heightened precipitation capture and enhanced water storage capacity in the soil (Jakab et al., 2019). These mechanisms have been observed in various studies reviewed by Basche et al. (2019) reporting substantial enhancements in infiltration rates associated with the presence of crop residues when compared to bare soil conditions. In addition, a systemic analysis conducted by Turmel et al. (2015) documented that the introduction of CCs in cropping systems with conventional tillage can mitigate or eliminate surface crusting and decrease surface runoff as well as soil loss, associated with tillage practices while improving water infiltration rates. Meanwhile, Blanco-Canqui and Ruis (2020) reported that CCs should be utilized to improve soil hydraulic properties in systems under no-tillage management, otherwise, it could not be expected an enhancement in soil hydraulic properties from no-tillage compared to conventional tillage. Similarly, a study conducted by White and Weil (2010) in a no-till rotation, demonstrated that rye (*Secale cereale* L.) CC enhanced soil water content at a depth of 0.2 m compared to no CC and no-tillage. Koudahe et al. (2022) in their critical review reported that residues from CCs left on the soil might contribute to improved rainwater infiltration while concurrently minimizing evaporative losses more than incorporated residue (especially in the cases of barley, rye, sorghum - *Sorghum bicolor* L. -, and sudangrass - *Sorghum drummondii*). In addition to managing residues, the timing of CC termination has been identified as a contributing factor influencing the water balance within cropping systems with CCs. Hence, earlier termination might reduce competition for water between the CC and cash crop compared to a termination right before the cash crop sowing, especially in arid climates or during periods of low rainfall (Alonso-Ayuso et al., 2018).

Soil erosion. Another positive effect related to an improved soil structure and soil hydraulic properties is the reduction of water and wind soil erosion. As reviewed by Van Eerd et al. (2023) the CCs serve as a protective shield for the soil, absorbing the energy of raindrops, mitigating soil aggregate detachment, enhancing soil surface roughness, fostering the development of water-stable aggregates, extending the duration available for water infiltration, delaying the initiation of runoff, intercepting runoff, and reducing runoff velocity. This cumulative effect of increased infiltration and decreased runoff during rainfall events significantly diminishes soil erosion as also reported by several studies reviewed by Blanco-Canqui et al. (2013) and Colazo and Buschiazzo (2010). Soil erosion by both water and wind disrupts the uppermost soil layer, which typically harbors substantial quantities of soil organic matter (SOM) and immobile nutrients critical for soil fertility. This substantial loss of topsoil is reported as the primary cause of soil degradation, affecting soil physical, chemical, and biological properties, as well as leading to decreased productivity of agricultural lands as reviewed by Lal (2015), and it stands as the foremost factor contributing to land deterioration (Dabney et al., 2001). For this reason, the positive effect of CCs in reducing this phenomenon is considered of crucial importance (Van Eerd et al., 2023).

Soil temperature. CCs canopy cover and residues are also reported to contribute significantly to regulating soil temperature in agricultural cropping systems (Yogi et al., 2022). Specifically, CCs have the capacity to lower soil temperature during the summer months and raise it during winter (Dabney et al., 2001; Kahimba et al., 2008). The reduction in soil temperature during summer seasons might lead to decreased evaporation and increased soil water retention. However, this effect may be disadvantageous in cooler regions where crop growth is constrained by lower temperatures (Dabney et al., 2001). Nevertheless, the influence of CC residues on soil temperature is subject to variation contingent upon seasonal changes, diverse tillage systems, CC species, and the extent of surface residue coverage (Blanco-Canqui et al., 2015).

2.2 CC effects on soil chemical properties

Chemical indicators have conventionally served as the primary metric for evaluating soil quality. Agricultural practitioners routinely assess their soil's nutrient status and administer essential nutrients, particularly nitrogen (N), alongside phosphorus (P), potassium (K), sulfur (S), among others, to facilitate optimal crop growth. However, SOM stands out among various indicators as a crucial and widely utilized measure, integral to nearly every soil quality assessment (Vasu et al., 2020). Hence, SOM and soil nutrient levels have been identified as the top two primary indicators for evaluating soil quality, with SOM ranking as the foremost indicator followed by nutrient levels (Hijbeek et al., 2017).

SOM and C. The significance of the SOM in enhancing soil fertility and productivity has received extensive recognition within the literature (Lal et al., 2015; McLauchlan, 2006). SOM plays a critical role in stabilizing soil aggregates, facilitating tillage practices, enhancing soil aeration, and augmenting both water retention and buffering capacities (Zuber et al., 2018). Additionally, the decomposition of SOM contributes significantly to the release of essential nutrients that become readily available to plants (Gmach et al., 2019). Furthermore, the accumulation of SOM and the retention of carbon (C) within soils, which represent the largest C reservoir globally, offer substantial aid in counterbalancing C emissions (Lal, 2015). The depletion of SOM in agricultural systems has been widely observed, especially in conventional agricultural systems relying on tillage practices (Evans et al., 2016; Sanchez de Cima et al., 2015). These tillage operations have been observed to expose protected C to microbial activity, accelerating mineralization and the production of carbon dioxide (CO_2), consequently resulting in C losses (Evans et al., 2016; Sanchez de Cima et al., 2015). The SOM depletion has intensified challenges in plant production (Dungait et al., 2012). In this context, CCs could represent a mechanism aimed at enhancing the input of OM into soils, particularly in agricultural systems reliant on tillage (Plaza-Bonilla et al., 2016).

A scientific review conducted by Blanco-Canqui et al. (2015) highlighted that the extent of SOC accumulation is contingent on site-specific factors and varies based on several elements, among which the CC species, the input of biomass from CCs, duration of CCs implementation, the tillage practices, soil texture, and the initial soil C levels, and climatic conditions. However, overall, an increase in the SOC after CC introduction has been observed in several studies and meta-analyses conducted in different climatic conditions and cropping systems (Ding et al., 2006; Frasier et al., 2016; Harasim et al., 2016; McDaniel et al.,2014).

Diverse CC species differently affect SOC accumulation. Mazzoncini et al. (2011) observed that leguminous CCs led to a higher accumulation of SOC compared to non-leguminous CCs or control, both 5 and 15 years after the beginning of their experiment. Conversely, Blanco-Canqui et al. (2013) observed that grass CCs exhibit higher efficacy in elevating soil C levels in the long-term period compared to leguminous CCs, primarily attributed to the slower decomposition rates of grass CC residues. The utilization of mixtures, comprising various CC species, tends to enhance SOC content significantly more than the use of single-species CCs, primarily due to increased production of both above- and belowground biomass (Chapagain et al., 2020). Numerous studies conducted across various climatic conditions have consistently demonstrated that, among diverse CC species, the use of hairy vetch (Finney et al., 2017) crimson clover (*Trifolium incarnatum* L.) (Khan et al., 2021), pea (*Pisum sativum* L.), turnip Brassica (Olson et al., 2014), oats, and rye (Stavi et al., 2012) have notably augmented SOC concentrations when compared to plots without CCs.

CCs can bolster SOM levels largely due to the input of their residues, whether retained on the soil surface or incorporated into the soil matrix (Ghimire et al., 2017). While the addition of nutrients by chemical fertilizers offers logistical advantages concerning storage, application, and management, they do not contribute to augmenting SOM levels to the extent achievable through the incorporation of plant residues. Both aboveground and belowground CCs biomass residues have been extensively reported to contribute to increasing the SOM (Blanco-Canqui et al., 2013; Poeplau and Don, 2015), however, belowground inputs have been observed to exert a greater impact owing to their prolonged persistence and physical entrapment in rhizodeposits compared to aboveground biomass, which is more readily decomposed (Tiemann and Grandy, 2015).

When coupled with no-tillage the CCs seem to perform even better in terms of SOC storage. Franzluebbers (2021) found that no-tillage plus CCs provided two times more C storage than no-tillage alone, reflecting the combination of minimum soil disturbance and CCs increasing soil C. Similarly, Blanco-Canqui et al. (2013) reported that, in general, the inclusion of CCs in no-tillage systems leads to an extra SOC accumulation (in the first 0-20 cm depth) from 0.10 to 1 Mg ha^{-1} year^{-1} compared with no-tillage systems without CCs. Furthermore, the CCs benefits become discernible more rapidly under no-tillage due to decrease rate of residues decomposition compared to conventional tillage management (Olson et al., 2014).

SOC accumulation with CCs varies with soil textural class. Notably, the soil's ability to retain and safeguard SOC exhibits a positive correlation with the clay content within the soil (Rasmussen et al., 2018).

Also, eroded soils with low initial C levels can have a greater capacity to accumulate C with CCs, compared to soils with initial higher SOC levels as reported in two meta-analyses conducted by Poeplau and Don (2015) and Ruis and Blanco-Canqui, (2017).

Among different mechanisms, CCs can also foster SOM storage in the soil, reducing its potential loss through erosion phenomena (Blanco-Canqui et al., 2015).

In addition, the quantity of precipitation influences both the CCs' biomass production and the C inputs they contribute to the soil. In semiarid regions (< 500 mm), despite the potential for SOC increment in association with CCs, the lower levels of rainfall lead to reduced biomass production. Consequently, the accumulation of SOC might take an extended period to materialize compared to regions experiencing higher precipitation levels exceeding 500 mm (Blanco-Canqui, 2023).

Soil N cycle. The interaction among different N forms within the soil, plant, animal, and atmosphere collectively constitutes the overall N dynamic. The soil N cycle is influenced by factors such as plant species, type (organic, inorganic, organo-mineral) and quantity of fertilizers applied, microbial activity rates, soil's physical and chemical properties, climatic conditions, and agricultural practices specific to a region. The utilization of CCs significantly modifies N dynamics and increases its mobility in the agroecosystem (Ramdhane et al., 2019). During their developmental phase and subsequent decomposition, CCs possess the capacity to influence the accessibility of plant nutrients within the soil, particularly N (Eichler-Lobermann et al., 2008). Similarly, to what was reported for the SOC, the influence of CCs on the N cycle is contingent upon multiple factors such as the CC species and the duration of their implementation, the quality of the CCs residues, as well as their sowing and termination time and type (Shelton et al., 2018), the climatic conditions, and soil attributes (Yang et al., 2021).

Looking at different CC species it has been reported that leguminous CCs can engage in atmospheric N_2 fixation and establish symbiotic relationships with *Rhizobium* bacteria capable of converting atmospheric N_2 into organic N (Pandey et al., 2017; Tonitto et al., 2006), thereby augmenting soil N content. Studies reviewed by Sainju et al. (2002) demonstrated a significant increase in N concentration in soils after the termination of hairy vetch and crimson clover compared to fallow soils. Differently, non-leguminous CCs actively scavenge and recycle N from the soil for their development, leading to rapid sequestration of nitrate (NO_3^-) from the soil, thereby reducing its potential environmental loss (Kaye and Quemada, 2017). Wendling et al. (2016) specifically documented that under favorable conditions, CCs can scavenge as much as 120 kg N ha^{-1} within a mere 3-month period. Recently, the adoption of CCs with minimum tillage has gained popularity in conservation agricultural systems as an effective means to mitigate N loss through N cycling (Nitu et al., 2021). N loss resulting from agricultural activities significantly contributes to groundwater and surface water contamination, and it has been reported that the presence of CCs, rather than fallow soils can significantly reduce the agricultural fields' susceptibility to leaching and surface water runoff (Plaza-Bonilla et al., 2015). Numerous studies conducted across different countries such as the United States (Dozier et al., 2017), France (Couedel et al., 2018), Ireland (Hooker et al., 2008), and England (Cooper et al., 2017) confirmed the effectiveness of non-leguminous species (including oilseed radish, mustard - *Sinapis alba* L., and cereal rye) in diminishing NO_3^- leaching. A meta-analysis conducted on irrigated cropping systems indicated that non-leguminous CCs mitigated NO_3^- leaching by 50% compared to both leguminous CCs and fallow soil (Quemada et al., 2013). Furthermore, multiple investigations consistently underscored the capacity of non-leguminous including rye, oats, barley, and ryegrass (*Lolium multiflorum* L.) to predominantly scavenge NO_3^- and other essential nutrients in contrast to leguminous CCs (Dabney et al., 2010; Quemada et al., 2013). Nielsen et al. (2015) also reported that canola and forage radish have shown superior potential for extracting residual soil NO_3^- compared to leguminous CCs. A comprehensive analysis conducted by Kaspar and Singer (2011) on several studies evaluating the management implications of CCs revealed notable reductions in NO_3^- leaching ranging from 6% to 94% with specific species. The author reported hairy vetch and purple vetch (*Vicia benghalensis* L.) as those with the lowest percentage of reduction, whereas rye, ryegrass, oats, winter wheat, and mustard with the highest. This ability to reduce the NO_3^- leaching by 40-70% during the winter season of non-leguminous species compared to fallow land, might also increase the N accumulation in the range of 20-60 kg N ha^{-1} after their termination, as reported in many studies reviewed by Tonitto et al. (2006). However, the potential impact of CCs on controlling NO_3^- leaching could vary based on their sowing and termination dates. Effective management of NO_3^- leaching through CCs might be compromised if the CC is sown late (Teixeira et al., 2016) or if a winter-killed CC is utilized (White et al., 2020). Additionally, Ramdhane et al. (2019) observed higher total N and C content when CCs were terminated due to frost compared to termination through rolling or glyphosate application.

Following the termination of CCs, the availability of organic N within the CC biomass—whether acquired through scavenging or biological N2 fixation—is contingent upon their residues' C:N ratio. Generally, N immobilization occurs when the C:N ratio is $\geq$ 26, while mineralization occurs at a ratio $<$ 13 (Justes et al., 2009). When C:N ratio is between 13 and 20, net N immobilization occurred during the first weeks after termination, followed by a subsequent re-mineralization. The C:N ratio is influenced by species and maturity, resulting in each CC or blend exhibiting a distinct C:N ratio. Leguminous CC species typically possess a low C:N ratio due to their high N accumulation and low tissue C content. Consequently, this characteristic leads to a more substantial supply of N during decomposition from leguminous CCs than from non-leguminous species (Di Palo and Fornara, 2017).

Grass species characterized by high C:N ratios undergo slow decomposition processes leading to the immobilization of soil N. Consequently, this circumstance may potentially necessitate higher application rates of N fertilizers to attain optimal economic yields in subsequent cash crops such as cotton, corn, and sorghum (Dabney et al., 2001, 2010). Differently, leguminous species are reported to be able to provide an equivalent of N fertilizers which ranges from 50 to 150 kg ha^{-1} (Crandall et al., 2005; Seo et al., 2000). Other studies confirmed similar results suggesting that leguminous CCs can supply significant quantities of plant-held N to subsequent crops (Blanco-Canqui et al., 2011; Thilakarathna et al., 2015), thereby, increasing soil fertility and potentially reducing N fertilizer requirements of the subsequent cash crop (Fortuna et al., 2008). The leguminous composition (low C:N ratio) favor higher mineralization activity compared to grass species which might contribute to the pool of plant available N (Dabney et al., 2001). In addition, when the leguminous species are used in a mixture with cruciferous, they have been shown to increase N mineralization due to a reduced C:N ratio compared to a sole cruciferous CC (Couëdel et al., 2018). However, some studies reported that winter leguminous CCs demonstrated an increase of soil N only after 5 years since their first introduction in the cropping system (Sainju et al., 2003; Villamil et al., 2006). Assessments of N input have been frequently based only on the breakdown and N contribution of shoot residues while N inputs of roots have mostly been overlooked (Jani et al., 2016). However, roots might account for about 30% to 50% of total CC biomass (according to specific species) and can significantly contribute to the increase of soil N even if their decomposition and N release is usually slower compared to the shoots (Sainju et al., 2002). Nevertheless, Jani et al. (2016) in the case of leguminous CCs (pea, clover, and vetch - *Vicia sativa* L.) reported a rapid root decomposition and N release comparable of that of their aboveground biomass. The lower C:N ratio typical of leguminous residues, if on one hand might release higher N quantity in a shorter time compared to the grass species (Dabney et al., 2001) on the other hand limits the persistence of the plant residues on the soil surface, which reduces benefits linked to surface residues. Besides the CC species, the management of the CCs sowing, and termination might significantly affect the N dynamics. Studies indicate that a shorter growing period (Kaspar and Singer, 2011) and early spring decomposition of winter-killed CCs may be less effective in preventing N leaching compared to CCs overwintered (Dean and Weil, 2009). However, this outcome is contingent upon the winter and spring precipitation patterns, which determine the conclusion of the drainage period and consequently, the risk of N leaching.

There exists a possibility that the decomposition of CCs in early spring could potentially enhance N supply to subsequent cash crops without posing substantial risks associated with N leaching (Dean and Weil, 2009).

Other macro and micronutrients. The CCs introduce additional C in the soil which serves as an energy source for soil microorganisms and enzyme activity (Dube et al., 2014) which facilitates the efficient cycling of various macro and micronutrients essential for crop development (Alloway, 2008). Additionally, different studies observed that the increase in SOM caused by the CCs introduction in a cropping system enhances the soil's cation exchange capacity, thereby improving its ability to retain essential elements such as N, S, and P (Du et al., 2014), as well as macronutrients like calcium (Ca), K, and magnesium (Mg) (Alloway, 2008). Studies reviewed by Alloway (2008) and Dube et al. (2014) observed an enhanced availability of some micronutrients (zinc - Zn; copper - Cu) for plant uptake in treatments with CCs compared to fallow soil. The authors suggested that the creation of complexes between SOM and micronutrients increased the plant availability of the latter.

Considering P, the macronutrient that is receiving increasing attention due to imminent concerns regarding its diminishing availability in the foreseeable future (Zou et al., 2022), CCs are utilized to enhance the efficiency of P utilization from added organic or mineral fertilizers by fostering increased soil biological activity or by facilitating the uptake and preservation of soluble mineral P in soils that strongly fix P (Kuo et al., 2005). This practice augments the soil microbial community by leaving behind a legacy of augmented mycorrhizal abundance, P content in the microbial biomass, and enhanced phosphatase activity (Hallama et al., 2019). However, a long-term rotation involving soybean (*Glycine* max L., Merr.) and ruzigrass (*Urochloa ruziziensis*, R. Germ. & C. M. Evrard Crins) resulted in reduced soil P availability. This reduction occurred due to diminished P mobility, irrespective of P application rates, by decreasing P diffusion and resupply from the solid phase of the soil. Such changes may significantly impact crop production (Almeida, 2019).

2.3 CC effects on agroecosystem biological components

Microbial population and activity. The increase in biological activities serves as a dynamic indicator of enhancements in soil properties and the comprehensive ecosystem services it offers (Blanco-Canqui et al., 2015). The utilization of CCs stands as a widely adopted strategy aimed at fostering favorable environmental conditions conducive to the proliferation of both macro- and microorganisms within agricultural soil. However, the biological properties of soil exhibit significant dynamism and susceptibility to numerous influencing factors, thereby presenting challenges in isolating specific effects for analysis.

Consequently, numerous studies have been conducted globally under diverse environmental conditions to investigate the potential impact of CCs on the activity, abundance, and diversity of soil organisms, including, nematodes, protozoa, fungi, and bacteria (FAO, 2021; USDA, 2019). These studies aim to discern the nuanced effects and interactions of CCs on soil biota across varying ecological settings. Through the addition of OM to agricultural soils, the CCs serve as a vital source of energy and nutrients, stimulating microbial growth and activity (Abbasi et al., 2015). The microbial biomass (predominantly composed of bacteria and fungi) alongside soil microfauna and algae (Musbau et al., 2021), assumes a crucial role in the dynamics of SOM and nutrient processes by serving as both a reservoir (during immobilization) and a contributor (through mineralization) of essential plant nutrients (Smith and Paul, 2017). CCs have been extensively documented as capable of creating favorable environmental circumstances, encompassing moisture levels, temperature, and the availability of C, conducive to the proliferation of soil microorganisms (Murungu et al., 2011). Microorganisms actively discharge enzymes that catalyze and enhance multiple biochemical reactions (Kujur and Kumar Patel, 2014), fostering the decomposition of crop residues, facilitating nutrient cycling, and enabling the release of inorganic nutrients essential for plant growth (Baležentienė, 2022). Hence, the activity of microorganisms and enzymes stands as the primary bridge connecting SOM to the release of nutrients for plant utilization. Although a broad association exists between CCs, microbial communities, nutrients cycling, and soil health, potential species-specific effects have been observed and warrant further investigations (Finney et al., 2017). Both during the CCs growth, through the root's activity, and after the CCs termination through their residues, different species of CCs might differently affect the soil microorganisms (Lehman et al., 2014).

Certain studies highlight the primary impact of CCs on the overall abundance of microbial populations (Rankoth et al., 2019). Conversely, other research indicates that specific species of CCs tend to promote particular functional groups within the microbial community both during their growth and after their termination (Finney et al., 2017). The cultivation of CCs such as crimson clover, pea, and vetch has shown an increase in microbial biomass C, arbuscular mycorrhizal fungi population, bacteria population, and soil enzyme activities in comparison to control conditions, exhibiting variations across diverse environmental and management practices (Blanco-Canqui et al., 2015; Mukumbareza et al., 2015). Similar results were also observed in other studies, which revealed an augmented population of arbuscular mycorrhizal fungi and enhanced microbial diversity in treatments with crimson clover, oats, and hairy vetch CCs compared to fallow controls without CCs (Benitez et al., 2016; Detheridge et al., 2016). Additionally, multiple investigations have reported a notable increase in both bacteria population and diversity, accompanied by enhanced crop yields in soils amended with rye, alfalfa (*Medicago sativa* L.), oilseed radish, and hairy vetch (Fernandez et al., 2016; Lupwayi et al., 2021). Furthermore, leguminous CCs exhibit the capacity to fix atmospheric N_2 during their growth phase via a symbiotic relationship with soil bacteria (Rhizobia). Calderon et al. (2016) observed that the dynamics of the soil microbial community exhibited a strong correlation with soil moisture levels besides the presence of living plant roots during the CCs growing season. This observation suggests that in environments characterized by limited water availability, the conservation of soil moisture might hold greater significance than intensifying cropping practices in fostering favorable soil biological conditions.

After the CCs termination, the incorporation of both above and belowground biomass into the soil fosters an increased microbial activity, and enzymatic processes, mostly related to the augmentation of SOM and the enhancement of soil physical properties such as soil aggregation, aeration, water infiltration, and porosity (Blanco-Canqui et al., 2011). Nonetheless, different CC species as well as the type of residue management alters in a different way the soil microbial communities and their structure (Finney et al., 2017). Existing literature underscores the key role of the C:N ratio of CC residues integrated into the soil, serving as a primary determinant controlling microbial activity and the dynamics of mineralization and immobilization processes (Abbasi et al., 2015; Brennan et al., 2017). Comparative studies have reported fewer bacterial and fungal populations on grass residues compared to leguminous residues (Reddy et al., 2020). Furthermore, investigations indicate higher populations of soil microorganisms in residue mulch compared to incorporated residues (Liu et al., 2020).

Earthworms. In addition to beneficial soil fungi and bacteria, multiple investigations have delved into the impact of CCs on earthworm activity (Euteneuer et al., 2020; Roarty et al., 2017). Soil earthworms play a significant role in enhancing soil aeration, facilitating OM decomposition, nutrient cycling, and the breakdown of microbial biomass (Amador and Gorres, 2007). Diverse and opposite results have been reported about the effect of CCs on earthworms. However, the majority of them observed that the utilization of CCs might foster the presence of earthworms in the soil, particularly with prolonged use, increasing the presence of plant residues in the soil, enhancing the soil nutrient levels as well as improving soil structure (Blanco-Canqui et al., 2011; Korucu et al., 2018). The quality of CC residues might affect soil earthworms. Specifically, leguminous CCs have exhibited a greater capacity to support increased earthworm populations compared to grasses and brassicas (Pelosi et al., 2015; Roarty et al., 2017). In some studies, regardless of the species, it has been reported that the CCs contribute to an increase in the abundance of earthworms (Roarty et al., 2017; Stobart et al., 2015), whereas they do not necessarily impact the number of earthworm species present (Blanco-Canqui et al., 2011; Korucu et al., 2018). Differently, some researchers revealed no significant differences of earthworm abundance after CCs introduction compared to control treatments without CCs (Ashworth et al., 2017; Sanchez de Cima et al., 2015; Stroud et al., 2017). Among the reasons why it is reported that the tillage practices commonly employed for CC incorporation or throughout crop rotations for establishment might diminish earthworm populations (Crotty et al., 2016; Perego et al., 2019). Additionally, the life cycle of the earthworm species *Lumbricus terrestris* spans around 6 months under favorable conditions and this potentially implies that study durations might not adequately capture the entire lifecycle of earthworms following CC treatments. Moreover, the spontaneous vegetation and weeds present in control plots can serve as alternative food sources for earthworms (Ashworth et al., 2017). Besides these factors, it is also reported that the earthworms' activity depends strongly on soil type, crop management, and climatic conditions (Euteneuer et al., 2020).

Pest and wildlife biodiversity. Crop rotation strategies involving CCs enhance agricultural diversity, thereby interrupting pest and disease cycles within cropping systems. Additionally, CCs might contribute to disease suppression by fostering specific segments of the existing soil microbial community. This phenomenon is linked to the interactive influences of root exudates and the affinity of various crops' roots towards beneficial organisms (Singhal et al., 2020). Single CCs species and their diverse mixtures might also exhibit allelopathic effects, serving to mitigate pest infestations besides controlling weeds (Treadwell et al., 2007). Moreover, after the CCs termination, the incorporation of their residues into the soil can potentially diminish soil-borne diseases and disrupt habitats for insects (Adetunji et al., 2020). Several studies have demonstrated that CCs such as rye, velvet bean (*Mucuna pruriens* L.), hairy vetch, and sorghum-sudangrass have led to an increase in predator populations, including syrphid flies and ladybird beetles, consequently regulating pestilent aphids and reducing pesticide usage (McNeill et al., 2012). Furthermore, employing CCs as trap crops in corn cultivation has proven effective in controlling corn earworm and tarnish bugs, while also aiding in the long-term reduction of soil-borne pathogens (Hoorman, 2009). Various CCs offer advantages in accommodating beneficial insects beyond controlling pest populations. Several flowering CCs, such as legumes, clovers, or buckwheat (*Fagopyrum esculentum* L.), possess substantial value in attracting insects and other animals. They also play a pivotal role in drawing pollinators, thereby enhancing crop field pollination rates (Sharma et al., 2018a). Crimson clover, for instance, has been documented to provide honeybees with nectar, serving as an attraction for pollinators (Reddy and Reddy, 2017).

Weeds. Well-designed CC systems have the potential to reduce the usage of herbicides and labor for weed control, providing farmers with a cost-effective strategy for managing weeds, a pivotal determinant in their adaptive agricultural practices (Tataridas et al., 2022). CCs exert weed suppression through various mechanisms, including resource competition for nutrients and water, shading, disruption of ecological niches, and the release of phytotoxins via both root exudates and decomposing residues. These actions collectively minimize weed seed banks and impede the germination, growth, and reproduction of weeds (Fageria et al., 2005; Kruidhof, 2008; Moonen and Barberi, 2006). CCs actively outcompete weeds during their growth phase, and their residual components contribute to further weed suppression (Fernando and Shrestha, 2023). The effectiveness of this suppression is notably influenced by several factors, including plant density, initial growth rate, aboveground biomass, duration of leaf area, persistence of residues, and the timing of subsequent crop planting (Kruidhof, 2008; Linares et al., 2008). Effective weed control is typically achieved through dense CCs plantings and allowing these CCs to grow for extended periods (Kruidhof et al., 2008). Although most CCs generally exhibit weed-suppressing properties, grass species may offer more effective early-season weed control compared to legumes due to their earlier germination and quicker root system development (Lundkvist and Verwijst, 2011). Grasses such as rye, sorghum, and sorghum-sudangrass demonstrate effectiveness in weed suppression through the release of natural substances that hinder the growth of neighboring plants ('*allelopathy*') (Treadwell et al., 2007). Annual CCs can serve as a means to manage perennial weeds, especially if they effectively shade out these weeds just before the weeds begin to replenish their storage organs, such as rhizomes (Teasdale et al., 2007). However, in organic systems utilizing repeated annual CC applications alongside no-tillage practices, control of grassy weeds was not observed (Treadwell et al., 2007). Reduced weed biomass was observed with an annual clover compared to perennial clovers used as spring CCs (Meiss et al., 2010). Similarly, Ross et al. (2001) observed that seven clover species demonstrated weed biomass suppression, although the effectiveness varied among different species and management practices.

The suppression of weeds by CCs residues after their termination hinges upon effective soil coverage, which can typically be sustained for a period ranging from 30 to 75 days. This duration is contingent upon the decomposition rate linked to the quantity and biochemical attributes of the residues, rainfall patterns, soil temperature, and the vigor and pressure exerted by weeds (Ruffo and Bollero, 2003; Teasdale et al., 2004). However, the incorporation of residues into the soil diminishes their capacity for weed suppression due to heightened light exposure, the relocation of dormant seeds to the soil surface, and increased breakdown and dilution of allelochemicals (Nichols et al., 2015; 2020). Notably, residues of rye and barley have demonstrated efficacy in suppressing broadleaf weeds, whereas residues of hairy vetch have been observed to enhance weed growth (Mohler et al., 2018). This discrepancy might be associated with the nutrient-releasing properties of hairy vetch residues (Teasdale et al., 2007). Dabney et al. (2001) reported that residues from rye serve as highly effective mulches and have been documented to suppress weed growth for a period of up to 6 weeks subsequent to rye desiccation. Conversely, Mohler et al. (2018) reported that rye residues were found to be less effective in suppressing grassy weeds. The breakdown of Brassica residues instead, which contain glucosinolates, leads to the production of bio-toxins, including isothiocyanates. These compounds offer (partial) control over diseases, weeds, and parasitic nematodes (Weil and Kremen, 2007). The use of CC mixtures with complementary canopy characteristics (e.g., rye and clover) and differential root traits (e.g., fibrous vs deep tap roots) will provide superior CC performance and thus, more effective weed control (Drinkwater and Snapp 2007b; Linares et al. 2008).

2.4 CC effects on cash crops production

Understanding the repercussions of CCs on the final cash crop yield is a key point for adopting CCs (Kaspar and Singer et al., 2011), considering that economic viability is a crucial factor of any agricultural practice. CCs have exhibited varying impacts on subsequent crop yields, as reported in numerous studies summarized in several scientific reviews (Abdalla et al., 2019; Blanco-Canqui et al., 2015; Daryanto et al., 2018; Fan et al., 2021; Marcillo and Miguez, 2017; Ruis and Blanco-Canqui, 2017; Tonitto et al., 2006). All these studies, conducted across diverse geographic regions, encompassing various climatic conditions, and involving different combinations of CCs and cash crop species, have yielded conflicting and diverse outcomes encompassing the increase, reduction, or absence of effect on cash crop yields. The high variability in the crop response to the CCs introduction can be contingent upon various factors, including the: climatic conditions, CC species, CCs growing season, tillage system, duration of CC utilization over years, different cash crop types, and management practices associated with cash crops. In addition, in general, cash crop yields are correlated with the improvements in the soil's physical, chemical, and biological properties related to the introduction of the CCs (Tonitto et al., 2006). A key aspect of the CCs' effect on cash crops is the time of CC utilization. Numerous studies have reported that while CCs may not initially enhance crop yields in the first year, they exhibit positive effects over time (Bundy and Andraski, 2005). Positive yield and enhanced N uptake responses have often been observed in cereals following leguminous CCs compared to cereal monocultures, as shown by several studies reviewed by Marcillo and Miguez (2017). Additionally, as reported in the meta-analysis conducted by Kakraliya et al. (2018) the higher N content present in the soil after leguminous CCs compared to non-leguminous plays a pivotal role in determining the better response of ensuing cereals crops. This positive response, especially in terms of N uptake of the subsequently grown cereals is attributed to various factors, including the transfer of biologically fixed N from the legume, N sparing due to the presence of the antecedent legume, and reduced NO_3^- immobilization during the decomposition of leguminous residues (Dabney et al., 2010; Marcillo and Miguez, 2017). Nevertheless, the enhancement in grain yield might not solely be attributed to the quantity of accessible soil N. Enhancements in soil structure, disruption of pest and disease cycles commonly associated with cereal monoculture, as well as the phytotoxic and allelopathic effects originating from diverse crop residues, have all been implicated as contributing factors in the observed yield response (Blanco-Canqui et al., 2015; Ruis and Blanco-Canqui, 2017). In addition, CCs have the potential to augment crop yields in soils by significantly enhancing SOC, soil N, and overall soil properties over the long term. Blanco-Canqui et al. (2012) observed a correlation between crop yield and alterations induced by summer CCs in soil physical properties, concentrations of SOC, total N, as well as soil water content and temperature. This correlation exhibited greater strength in conditions without applied inorganic N compared to conditions with inorganic N application. These findings align with previous studies indicating a decrease in the benefits of CCs for enhancing crop yield as rates of inorganic N fertilization increase. A meta-analysis conducted by Miguez and Bollero, (2005) highlighted that the crop yield advantages derived from leguminous CCs diminish with higher rates of N fertilization.

Focusing on maize (*Zea mays* L.) and soybean cropping systems, which will be analyzed in detail within the present thesis, different results have been reported in the up-to-date literature about the effect of CCs introduction on their yield production. Looking at maize cultivation, recognized as one of the principal global food crops (USDA, 2019), there is a pressing necessity to develop more sustainable cropping systems centered around its production (Wojciechowski et al., 2023). Given its frequent cultivation within intensive systems characterized by tillage, monoculture, and short rotations resulting in bare soil during fallow periods, maize production relies extensively on synthetic inputs (e.g., fertilizers, pesticides), leading to various environmental concerns. Numerous studies have investigated the integration of CCs into maize cropping systems to address these challenges without risking of reducing maize final yield. However, global investigations have revealed conflicting outcomes regarding the effectiveness of CC integration in various maize cropping systems (Wojciechowski et al., 2023), and the same variety of results has been also observed in studies conducted in specific geographic areas. A meta-analysis encompassing studies conducted across the United States and Canada indicated a 21% and 13% increase in crop yield following the use of leguminous CCs and mixed species CCs, respectively, compared to scenarios with no CCs. However, it was noted that grass species CCs had no discernible effect on maize yield (Marcillo and Miguez, 2017). Conversely, another meta-analysis drawing from seven trials conducted in Europe reported a 3% decrease in crop yield subsequent to non-leguminous CC use (Tonitto et al., 2006). Both the above-mentioned meta-analyses (Marcillo and Miguez, 2017; Tonitto et al., 2006) concurred that in organic or reduced N farming systems, legumes exerted a greater positive influence on crop yield. Various individual studies either observed increased maize yield following CC use (Chen and Weil, 2011; DuPont et al., 2009; Kramberger et al., 2009) or found no significant effect on maize yield (Dozier et al., 2017; Gabriel and Quemada, 2011). Similar reductions in maize yield following CC use were documented in the temperate climates (Krstić et al., 2018). Unlike corn, CCs showed little effect on soybean yield (Bourgeois et al., 2022; Plaza-Bonilla et al., 2016). A meta-analysis conducted in temperate zones worldwide reported no significant differences in soybean yield after leguminous, non-leguminous, grass CCs or mixtures compared to fallow soils (Bourgeois et al., 2022). Similar results were also observed by Boselli et al. (2020) and Severini et al. (2021) showing that soybean yield collected after leguminous CCs (crimson clover and hairy vetch) as well as grass species (rye and triticale - *X Triticosecale*) and mixtures didn't show difference compared to a fallow control. Similar outcomes were also reported in the review conducted by Alvarez et al., (2017) in south America, and Marcillo and Miguez, 2017 in the United States and Canada were leguminous, non-leguminous and mixtures CCs were tested.

3. Cover crop selection and management

3.1 Species selection

The selection of CCs species is based on their suitability for the local pedoclimatic conditions, requisite services, existing crop rotation patterns, and alternative management strategies (Anderson et al., 2022; Cherr et al., 2006; Sustainable Agriculture Network, 2007). CCs embody several desirable traits that guide growers' choices, encompassing the capacity to: establish easily, rapidly develop to provide soil coverage, generate sufficient biomass in a short duration for residue maintenance, exhibit resistance to diseases without acting as hosts for cash crop diseases, facilitate straightforward termination, and demonstrate economic viability (Schomberg et al., 2010).

An initial fundamental consideration lies in acknowledging that the establishment and performance of any CC species or mixture can vary significantly based on local climates. Subsequently, it becomes crucial for farmers to prioritize among a range of essential services that a CC (or mixture) should aim to achieve. These priorities might encompass: providing N, retaining/recycling nutrients and soil moisture, mitigating soil degradation/erosion, sustaining or enhancing SOM levels, reducing pest incidences, and generating products and income (Cherr et al., 2006). Once the desired services provided by CCs are identified as priorities, a comprehensive assessment of the cropping system in which farmers intend to integrate the CCs should be conducted. This assessment should encompass an evaluation of crop rotations, commercial crop durations, inter-crop/fallow periods, tillage systems, and a thorough appraisal of potential risks related to pests and diseases of commercial crops. Moreover, in line with findings from various studies (Adetunji et al., 2020; Altieri et al., 2012; Sharma et al., 2018a), consideration should be given to several other parameters associated with CC species, such as their potential adaptation to environmental stressors like drought, flooding, low pH, nutrient deficiencies, and shading effects (live mulch); as well as the choice of species with complementary growth cycles, canopy traits, and root functionalities. Simultaneously, it necessitates the evaluation of potential adverse traits of CCs that might impact the associated cash crop. Additional factors to consider include the: (i) assessment of potential unfavorable residue properties, such as excessively high C:N ratios, coarse and recalcitrant residues impeding seedbed preparation, and allelopathic properties hindering initial germination and growth of subsequent commercial crops; (ii) competition with cash crops for light, land, water, nutrients, labor, and capital; (iii) weediness, excessive vigor/regrowth post-mowing or mechanical killing, and the potential to promote (host) pests and diseases; (iv) evaluation of the availability of affordable seeds, suitable equipment, techniques, and information ensuring optimal growth, termination, and overall system performance of CCs.

According to the seasonality, species such as winter rye, brassicas, hairy vetch, red clover and oats are reported among the most used cool season options while cowpea (*Vigna unguiculata* (L.) Walp.), brown hemp (*Crotalaria juncea* L.), soybean, sorghum perform well in warm seasons (Sharma et al., 2018b). If CCs are to be utilized in orchard settings, perennial species capable of self-seeding over multiple years become appealing. Conversely, their application in annual crop rotations necessitates the choice of annual species. Moreover, it is essential to opt for species that are well-suited and adapted to the climate and soil conditions of the designated area. In instances where species are intended for an annual rotation to substitute fallow periods, those adapted to early planting, capitalizing on autumnal growth, are desirable. If the region experiences continental influence, species resilient to cold temperatures are necessary to endure the winter's low temperatures.

When CCs are selected based on the intended purpose of managing the soil, they can be selected based on their ability in soil coverage, biomass production, allelopathic effect, C build-up and N_2 fixation. The utilization of CCs holds the potential for mitigating wind erosion, water erosion, and runoff as reported in the previous paragraphs; however, the degree of effectiveness varies based on the species of CCs due to inherent disparities in biomass coverage, residual height, root architecture, and decomposition rates (Balkcom et al., 2015; Wortman et al., 2013). Rapid-growth CCs with extensive root systems contribute to the augmentation of SOM at deeper soil layers (Dabney et al., 2001) and facilitate the formation of a porous framework within aggregate soil particles, thereby enhancing both water infiltration and retention capabilities (Darby et al., 2012). CC species endowed with fibrous root systems, such as ryegrass, rye, oats, and triticale, exhibit superior efficacy in curtailing soil erosion compared to those with thicker roots, like white mustard and fodder radish (De Baets et al., 2011). In addition, it is desirable for residue decomposition to occur slowly to prolong its surface presence and continue shielding the soil from erosion. Species that meet this requirement typically encompass a high quantity of fibers in their composition and possess a high C:N ratio (Quemada and Cabrera, 1997). Therefore, the selection of appropriate CC species capable of minimizing soil erosion serves as a pivotal strategy in ameliorating soil degradation and fostering enhanced soil fertility across diverse agroecosystems. These attributes also hold relevance for the objective of weeds control through resource competition. If the goal is nutrient capture, species with rapid establishment and growth rates are advantageous to exploit the autumnal growth period and promptly absorb available soil nutrients (Blanco-Canqui et al., 2015). To achieve this, a combination of strong root nutrient absorption and the ability to generate substantial biomass is of interest. Species possessing the described attributes may also be of interest during the CC growth period for weeds control, as competition will be intense. To augment SOM content and bolster soil quality, the selection of CCs type ought to prioritize those with a propensity for generating substantial quantities of biomass and/or root biomass (Balkcom et al., 2015). Research indicates a significant correlation between the use of high biomass-producing winter CCs and the marked increase in SOM (Dube et al., 2012). However, the biomass production of a given CC is subject to variation based on species (Wayman et al., 2015). In cases of adequate fertility levels, grasses typically outperform legumes in biomass yield, although variations in biomass production can exist even within the same species (Newman et al., 2007; Sainju et al., 2000). Moreover, it is imperative to consider the C:N ratio of CC residues to ensure adequate soil coverage while facilitating optimal decomposition, nutrient recycling, and requisite nutrient release rates. The deliberate choice of suitable CCs with enhanced weed-reducing attributes contributes to heightened soil water and nutrient availability. If the goal is to maximize the nutrients' release for the subsequent cash crop utilization, the focus lies on CCs with rapid residue decomposition hence, with residues having a low C:N ratio and low lignin content. For usage as green manure indeed N-fixing species are necessary. However, after the termination of these CC, the residue will remain on the surface for a shorter duration, resulting in reduced expected control of nutrient losses due to lower erosion control and lower competition with weeds.

When introducing CCs in a cropping system it is imperative to account for the specific soil conditions. While many CC species demonstrate adaptability to a wide spectrum of soils, species such as hairy vetch, crown vetch, crimson clover, sunn hemp, and alfalfa emerge as suitable choices for sandy, well-drained soils (Moncada and Sheaffer, 2010; Shekinah and Stute, 2018). Specifically, winter rye and annual ryegrass exhibit a preference for sandy or loamy soils, whereas brassicas such as mustard and rapeseed thrive in neutral soils (Moncada and Sheaffer, 2010). Sunn hemp, cowpea, crown vetch, spring oats, and buckwheat exhibit robust growth in moderate to low-fertility soils, positioning them as viable options for soil rehabilitation in depleted environments (Moncada and Sheaffer, 2010; Shekinah and Stute, 2018).

To date, among the different species identified in the literature as the most frequently used for CC purposes, the majority belong to two families: grasses and legumes. Among the grass family, for food and/or fodder (oat, rye, wheat, triticale*,* barley, ryegrass, sorghum). Due to their rapid establishment, vigorous growth, high biomass accumulation, and slowly decomposing residue (high C:N and significant fiber proportion), they are ideal species for erosion control, soil quality improvement, and C sequestration. Their competitive ability makes them suitable for weeds control. Moreover, some species like rye or ryegrass can contribute to weed suppression through the release of allelopathic substances that inhibit the emergence of specific species. Ultimately, they are highly adaptable species capable of withstanding stress conditions across diverse environments.

Within the leguminous family (hairy vetch, red clover., crimson clover, subterranean clover - *Trifolium subterraneum* L., common vetch, sweet yellow clover - *Melilotus officinalis* L., alfalfa, common pea, brown hemp) species traditionally used for food (e.g., soybean) or fodder (vetch, clover, etc.) can be found. Due to the N-fixing capacity of many leguminous species, they are primarily used as green manure. However, some of these species are well adapted and can establish quickly, accumulating biomass, making them considerable candidates for erosion reduction or weeds suppression.

Following grasses and leguminous are the Brassicaceae family (wild turnip, rapeseed - *Brassica napus* L., white mustard, mustard - *Brassica hirta* Moench). Some of these species have had traditional use as food or fodder, such as mustard, radish, or canola. These species are of interest due to their taproot system, which contributes to soil quality improvement by reducing issues like compaction, and they possess a high nutrient absorption capacity. If they endure winter temperatures and accumulate significant biomass in spring, they become suitable for use as capture crops.

Other species belonging to different families appear in the literature for various ecosystem services, although their usage is minor, such as buckwheat, or phacelia (*Phacelia* spp.).

Lastly, it's essential to note that for ease of use and adaptation, species known to farmers and readily available in the market for seed purchase are preferable. The CCs that are implemented can consist of a single species or be combined to form mixtures (Tosti et al., 2014; Finney et al., 2016; Murrell et al., 2017). Increasing biodiversity within the mixture allows for combining the attributes of these species, enhancing system resilience by reducing the risk of failure of any species due to extreme weather events, minimizing pest and disease risks, and bolstering some of their environmental services like N retention (Finney et al., 2016). A common mixture involves combining a cereal and a legume. This combination enables rapid establishment, initial coverage, and effective N retention during the fall-winter due to the competitive nature and nutrient-extracting capacity of the cereal. Simultaneously, the legume aids in N fixation, incorporating N that will be readily available for the subsequent crop (Gabriel et al., 2016)..

3.2 Cover crops' termination and residues management

Determining the optimal growth stage for terminating CCs stands as a pivotal management strategy, necessitating adaptable approaches contingent upon geographical location and specific cultivation objectives (Balkcom et al., 2015). The timing of CC termination is influenced by various factors such as the growing season, soil temperature, moisture levels, N management, as well as tillage practices for the subsequent cash crop cultivation (Balkcom et al., 2015).

Determining the most advantageous termination stage for CCs poses a challenge, often involving trade-offs among the diverse benefits they offer. For instance, CCs terminated at the mid-vegetative stage exhibit limited contribution to SOM accumulation and yield compared to late terminations (Hirpa, 2013; Njunie et al., 2004). This limitation arises due to constrained biomass production and rapid microbial degradation or loss of residue material through decomposition (Hirpa, 2013). Hence, the growth stage significantly influences both the quantity and quality of CC biomass, crucial factors governing the buildup of SOC and the potential availability or unavailability of N for subsequent cash crops (Alonso-Ayuso et al., 2014; Benedict et al., 2014). In a crop rotation system, early termination has demonstrated a reduction in the risk of competitive nutrient assimilation by CCs (Alonso-Ayuso et al., 2014). Notably, the N content of winter rye terminated at the flowering stage exhibited a 50% decrease compared to CCs terminated during the vegetative stage (Dabney et al., 2010). Postponing termination might also result in soil available N depletion owing to delayed mineralization of high C:N ratio residues (Thorup-Kristensen et al., 2010). However, delaying termination has also been reported to mitigate NO_3^- losses and enhance N use efficiency by capturing inorganic N prone to leaching into deeper soil profiles, subsequently releasing it in the topsoil through residue decomposition (Alonso-Ayuso et al., 2014). Nevertheless, the optimal termination timing for maximizing plant-available N varies depending on the type of CC (Balkcom et al., 2015). The availability of plant N derived from robust legume stands reaches its peak during the budding growth stage, gradually declining as the reproductive phase progresses (Sullivan and Andrews, 2012). Conversely, N availability from grass residues shows a positive trend from the early stages until the tillering phase, with a subsequent decline starting from stem elongation (jointing) (Sullivan and Andrews, 2012). Despite the wealth of information concerning CCs performance regarding biomass and N accumulation at maturity, constrained windows for planting commercial crops may necessitate premature termination of CCs (Cherr et al., 2006).

The impact of the termination date on soil water content results from a delicate equilibrium between the water extracted by actively growing CCs and the mitigation of soil water evaporation facilitated by the residue mulch (Dabney et al., 2010). Several studies suggest that while there might be a decrease in soil water content, delaying the termination of CCs enhances moisture conservation (Dabney et al., 2010). Conversely, earlier termination of CCs has been demonstrated to diminish preemptive competition for water, conserve soil moisture, and augment water accessibility for subsequent cash crops (Krueger et al., 2011; Stipešević and Kladivko, 2005). The water uptake by CCs can negatively impact the yields of subsequent dryland crops especially in semiarid regions (Obour et al., 2021).

The choice of the termination time can relate to the primary function of the CCs. If the primary objectives revolve around soil conservation and the accumulation of SOM, favoring older and more lignified residues might be preferable. However, such delays could potentially hinder the efficiency of rollers/crimpers. Additionally, older residues are more prone to interfering with planting equipment, and the resultant increase in the C:N ratio may heighten the risk of initial N immobilization. The use of herbicide or mowing serves to expedite residue decomposition and subsequent mineralization (Snapp and Borden, 2005). Farmers often contemplate delaying planting subsequent to residue termination to mitigate the risk of herbivores feeding on residues, potentially affecting the new crop. This delay allows for the settling of residues, facilitating subsequent planting operations, and prevents potential negative impacts of allelopathic compounds on the emerging crop (Fayad et al., 2020). An alternative approach involves placing seeds beneath the residue layer to minimize the risk of initial growth being impeded by allelopathic substances (Altieri et al., 2008).

Surface application of residues tends to favor saprophytic decomposition by fungi, while bacterial decomposition tends to prevail more with incorporated residues (Lal, 2015). Soil incorporation of CCs offers advantages such as increased soil residue contact and moisture buffering, which accelerates decomposition. Conversely, surface-applied residue might exhibit a higher propensity for N immobilization (Cherr et al., 2006). The rates of residue decomposition are contingent upon crop composition management, soil and climatic conditions (Snapp and Borden, 2005), and it might not align with the peak N demands of subsequent cash crop. Poor synchrony has been observed in numerous studies reviewed by Sarrantonio and Gallandt (2003) where nutrient release was either premature or delayed. The initial decomposition rates of residues largely hinge upon their C:N values and the presence of water-soluble and intermediate available C compounds (Cherr et al., 2006). In regions characterized by hot and humid conditions, early nutrient release from low C:N residue materials may result in substantial N-leaching losses (Cherr et al., 2007; Giller, 2001). Conversely, colder and/or drier conditions, coupled with the use of more recalcitrant residues and N-limited environments, can delay initial nutrient release, leading to net N immobilization that hampers the initial growth of commercial crops (Cherr et al., 2006; Sarrantonio and Gallandt, 2003). Achieving better synchronization between nutrient release and crop demand necessitates an enhanced understanding of residue decomposition and net mineralization. However, given the multifaceted influences of biotic, pedo-climatic, and management factors, the prudent utilization of decomposition models becomes essential for a comprehensive understanding of the interplay among these management factors.

4. Cover crops limitations and future directions

In addition to the previously mentioned potential positive impacts, the introduction CCs may also exhibit limitations and drawbacks. One primary limitation of CC practices lies in their considerable variability, with potential effects contingent upon various factors, including different species of CCs, diverse crop management strategies for both cover and cash crops, specific site characteristics, varying climatic conditions, and the temporal dynamics of their effects across different phases of crop rotations (Altieri et al., 2008; Cherr et al., 2006). Additionally, practicing CCs does not yield immediate beneficial outcomes; consequently, integrating them into cropping systems might lead to increased initial costs, labor requirements, and machinery utilization (Hoorman, 2009; Kaspar et al., 2008). In this context, there is an escalating demand for monitoring tools and methodologies, encompassing field measurements and model simulations, to evaluate the site-specific potential benefits of this practice. These tools should aim at refining both crop rotation design and CC management strategies according to site-specific necessities in order to reduce potential negative effects following the CCs introduction while maximizing their benefits (Altieri et al., 2008; Cherr et al., 2006).

Regarding the specific impact of CCs on soil structure, studies indicate that although CCs can enhance soil structure, measurable alterations may take up to four years to become markedly evident (Jokela et al., 2009). For example, investigations into the effects of CCs on soil bulk density over two years did not demonstrate significant differences in bulk density measurements (Chen and Weil, 2011; Sánchez de Cima et al., 2015). This lack of significant change could be attributed to the lateral displacement of soil particles by growing roots, resulting in minimal overall impact on soil bulk density (Chen and Weil, 2011).

Although CCs possess the capability to enhance soil hydraulic properties by improving soil aggregate stability and increasing soil microporosity, their influence on the soil water balance remains contentious. Notably, CCs have shown potential competition for water resources with subsequent cash crops, thereby reducing the available water for the primary crop (Alonso-Aluyo et al., 2018; Grabiel et al., 2016; Kramberger et al., 2009). This impact, combined with other effects on soil structure, may be specific to climatic conditions, and significantly influenced by CC species and management practices, including termination types and timing (Van Eerd et al., 2023). Global studies have highlighted that irregular yearly weather patterns within a particular climate zone can modify the impact of CCs on soil water, potentially diminishing water availability at cash crop sowing time. Investigations conducted in regions with annual rainfall ≥ 800 mm suggest that CCs, regardless of the species, can decrease soil water content before planting the cash crop (Krstić et al., 2018; Nielsen et al., 2016). However, diverse studies have reported specific effects contingent upon different CC species. Research in Poland by Harasim et al. (2016) indicates that, before establishing the cash crop, plowed bare soil fallow exhibited significantly higher soil water content than plots with white mustard CCs. Thelen et al. (2004) emphasized that moisture stress following rye CCs stands as a primary factor contributing to reduced soybean grain yields. Nielsen et al. (2016) observed that CCs capacity to deplete water resources for subsequent crops, consequently diminishing yields, is particularly emphasized in regions with low annual rainfall (< 500 mm). Yet, in semi-arid conditions (< 500 mm rain year^{-1}) in Austria, Bodner et al. (2008) observed that vetch and phacelia CCs demonstrated higher transpiration efficiency, measured as biomass production per unit of transpired water, compared to rye or mustard. However, also in areas experiencing higher precipitation levels (> 600 mm per year), treatments involving CCs may reduce soil water availability before establishing the cash crop when compared to treatments without CCs (Basche et al., 2016; Krstić et al., 2018). An investigation conducted in Serbia (rainfall of 610 mm year^{-1}) revealed that triticale significantly reduced soil water content more than vetch (Cupina et al., 2017). Conversely, in regions with higher rainfall (>1000 mm), White and Weil (2010) reported that CCs could lead to excessively moist soils, potentially impeding cash crop establishment. However, besides the weather conditions, the potential adverse impacts of introducing CCs on the soil water balance might be also related to specific CCs management (Van Eerd et al., 2023). Alonso-Ayuso et al. (2018) and Qin et al. (2021) observed that delaying the termination of CCs can result in competition for water with subsequent cash crops, potentially leading to a decline in final yield.

In addition to water competition, multiple studies have highlighted the phenomenon of 'pre-emptive competition' for N resources instigated by CCs. Particularly in arid regions with restricted cumulative drainage, CCs may compete for available N, potentially reducing the supply to subsequent cash crops and adversely impacting their yield (Macdonald et al., 2005). Investigations conducted in temperate regions suggest that grass species of CCs, such as rye and ryegrass, might decrease available N at cash crop sowing, while leguminous CC species can augment N availability (Couëdel et al., 2018). The potential adverse effects of CCs on the N cycle are also associated with N mineralization-immobilization processes linked to CC residues post-termination. The extent of N release from CC residues is significantly influenced by multiple factors, including residue quality, management practices, and environmental conditions (Poffenbarger et al., 2015), potentially leading to short-term N immobilization rather than providing additional N contributions (Rosolem et al., 2018). Excessive immobilization of N within CC residues, leading to potential N deficiency for subsequent crops, often occurs when plant material with a high C:N ratio, typically above 25, is added (Lacey et al., 2020). This phenomenon is exacerbated under conditions of low soil N levels and has been observed in CC species such as rye, triticale, barley, and sorghum (Woodruff et al., 2018). Some studies recommend preventing grass species of CCs from reaching maturity when utilized as cover crops to avoid potential N immobilization. Legumimous, characterized by lower C:N ratios (below 20), are considered high-quality CCs that decompose rapidly and mineralize N more effectively compared to grass CCs (Dabney et al., 2001). However, some studies reported that winter leguminous CCs did not increase soil N in less than 5 years (Sainju et al., 2003; Villamil et al., 2006).

Besides the CCs species, it remains uncertain whether and to what degree CCs can fulfill the N requirements of cash crops and reduce reliance on N fertilizers (Wittwer and van der Heijden 2020). Yet, determining the actual available N quantity for subsequent cash crops poses considerable challenges (Buchi et al., 2015; Thorup-Kristensen et al., 2010). Therefore, the development of reliable tools to monitor the CCs' growth and estimate their possible contribution or immobilization of the N resources might be of utmost importance to evaluate both the advantages and disadvantages related to their adoption in specific sites. This might help the farmers to manage the CC introduction in their agricultural systems maximizing the potential benefit and reducing the potential adverse effects, especially related to potential cash crop yield depletion (Snapp et al., 2005). A potential yield reduction after CCs introduction might be also related to potential negative effects caused by allelopathic substances or roots exudates released by CCs which might diminish the subsequent cash crop yield (Koehler-Cole et al., 2020). Current genetic research endeavors are concentrated on identifying CC genotypes that do not release these detrimental compounds (Griffiths et al., 2022). In addition, overall, cultivating CCs rarely causes pest issues. However, specific CCs may sporadically contribute to particular pest, disease, or nematode challenges in limited geographical regions, potentially by acting as an alternate host to the pest (Sustainable Agriculture Network, 1998). In addition, CCs have the potential to serve as alternate hosts for insects and pathogens during the offseason. Certain insect species and pathogens utilize CCs to complete their life cycles during the offseason, consequently emerging as significant pests for the subsequent main crop (Lu et al., 2020). In addition, CCs, especially non-leguminous (sorghum, pearl millet), have the potential of re-emergence if these CCs are not terminated properly. These re-emergence CCs compete with the main crop for space, light, water, and nutrients (Singh et al., 2016).

Similarly to what was observed for the soil structure improvement and the potential soil N increase after leguminous CCs, several studies have suggested that the utilization of

CCs did not yield a significant increase in SOC in the short term (Beehler et al., 2017; Sanchez de Cima et al., 2015). Several studies reviewed by Acuna and Villamil (2014) and Blanco-Canqui et al., (2014) demonstrated that while CCs generally contribute to the long-term increase in SOC, their effects might not be observable within the initial years following establishment. In addition, Kaspar et al. (2011) noted that the spatial variability within the soil and the naturally high inherent SOC content, make it challenging to quantify minimal changes in SOC resulting from CC usage within a short experimental timeframe. In semi-arid regions, although an increase in SOC due to CCs is observable, limited rainfall resulting in lower biomass production has been reported to prolong the accumulation process compared to regions experiencing higher precipitation (Ghimire et al., 2017). Yet, there is still great uncertainty about the efficacy of some practices, such as the use of CCs, because of highly variable effects on SOC accumulation (Chenu et al. 2019) due to site-specific climatic and soil conditions, as well as the accuracy of scaling up results from the microcosm to plot, field or even basin scale (Dignac et al. 2017). However, it is urgent in some parts of the world to increase SOC in degraded soils (SOC < 1%) in the short term (Tadiello et al. 2023). For this reason, it is necessary to develop integrated agronomic strategies able to increase the SOC stock within a short time that could be easily adopted by farmers, to increase their efficacy and scalability.

The integration of CCs into agricultural systems, aiming to maximize their beneficial effects while mitigating potential limitations, necessitates a methodical approach akin to a "trial and error" process. This entails the comprehensive incorporation of pertinent information about local pedo-climatic conditions into the decision-making process. Achieving the development of management practices and site-specific CCs-based cropping systems relevant to the local context may entail an extended timeframe spanning multiple years. The utilization of remote sensing techniques holds significant promise in discerning the growth dynamics and spatial distribution of CCs, particularly on a large scale. This application can assist farmers in adeptly managing CCs according to site-specific requirements. Notably, these remote assessments have garnered escalating attention and are advocated as an initial step in formulating effective policies to encourage CC adoption (Thieme et al., 2020). Researchers such as Hively et al. (2015) and Fan et al. (2021) have underscored the burgeoning significance of these remote sensing approaches. While remote sensing techniques serve as valuable tools for monitoring CC growth, the utilization of specific models becomes imperative to investigate the N contribution of CCs to subsequent crops.

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Objectives of the thesis

The present thesis aims to achieve four primary objectives. The first three objectives involve studying the impact of introducing two CCs successions - grasses followed by grasses; and grasses followed by leguminous and brassica species - within a 3-year maize-soybean succession experiment. The fourth objective explores the effects of introducing two CC successions - grasses followed by grasses, brassica followed by grasses - alongside organic fertilization matrices and irrigation within a 4-year maize-soybean crop succession in on-farm experimentation.

(i) The first objective aims to assess the influence of CCs on silage maize production and N dynamics in the short term. This involves analyzing maize N uptake, N use efficiency (NUE), soil nitrate content, and apparent soil N mineralization and immobilization processes (Chapter 1).

(ii) The second objective focuses on evaluating the effectiveness of utilizing time series vegetation indices (VIs) acquired from the Sentinel-2 satellite to monitor CC growth; and employing a simulation model to predict the N contribution of CCs to subsequent cash crops, while examining cash crop production and soil water content (SWC) (Chapter 2).

(iii) The third objective aims to investigate the effects of CCs on soil NO_3^-, soil N functional genes, and their relationships with SWC and cash crop yield throughout different phases of the crop rotation, aiming to comprehensively capture seasonal variability (Chapter 3).

(iv) The fourth objective seeks to evaluate the combined impact of CCs, fertilization matrices, and irrigation on the SOC stock in the short term (Chapter 4).

The detailed findings of the research conducted to achieve the four outlined objectives are presented in the subsequent four chapters.

Chapter 1

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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 pH/conductivity (soil/water solution with ration 1:2.5)
EC 1:2.5 (mS cm^{-1})	0.19 ± 0.02	
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:

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

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 $\text{Nmin}_{\text{sowing/harvest}}$ 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.

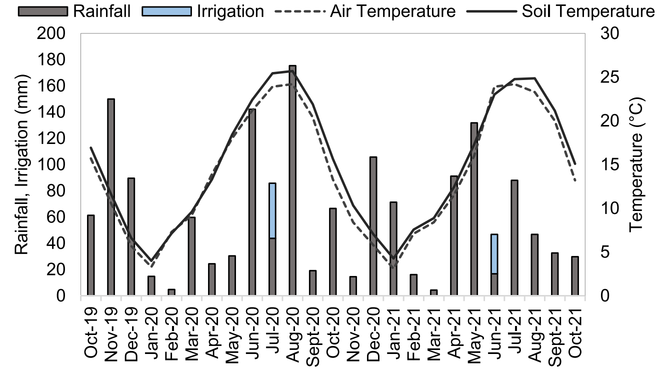

Fig. 1. Monthly mean temperature (air temperature and soil temperature at 20 cm depth) and cumulative rainfall, and irrigation events during the 2-year experimental period in Legnaro (Padova, Italy).

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).

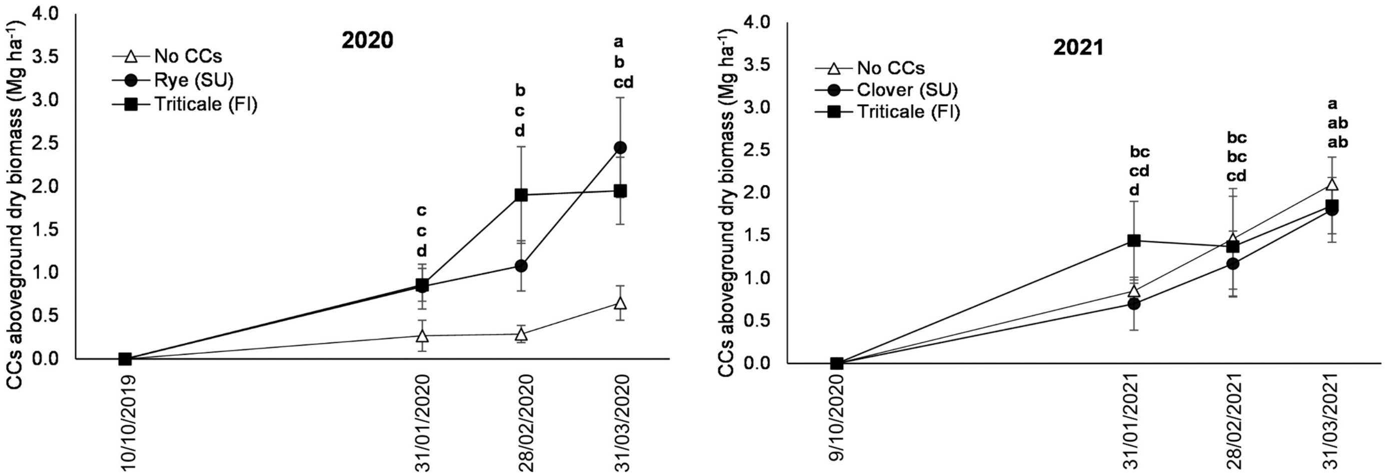

Fig. 2. Cover crops (CCs) aboveground dry biomass (Mg ha^{-1}) in 2020 and 2021 (average ± SE with 0.95 confidence interval, reported with the vertical bars). Different lowercase letters indicate significant differences among treatments and sampling dates within each year. Significance (p value ≤ 0.01)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

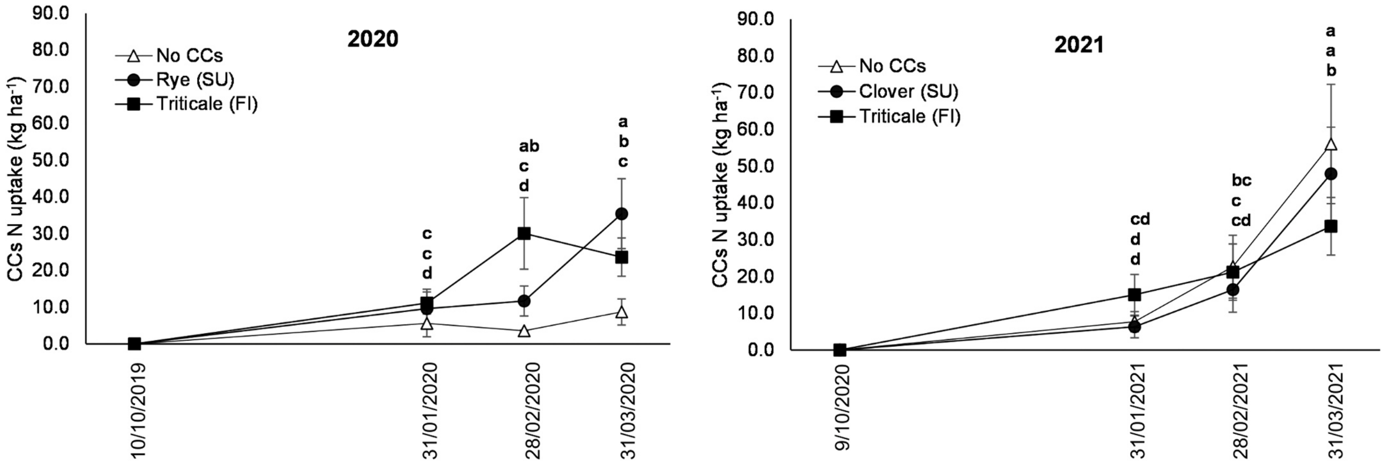

Fig. 3. Cover crops (CCs) aboveground dry biomass N uptake (kg ha^{-1}) in 2020 and 2021 (average ± SE with 0.95 confidence interval, reported with the vertical bars). Different lowercase letters indicate significant differences among treatments and sampling date within each year. Significance (p value ≤ 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

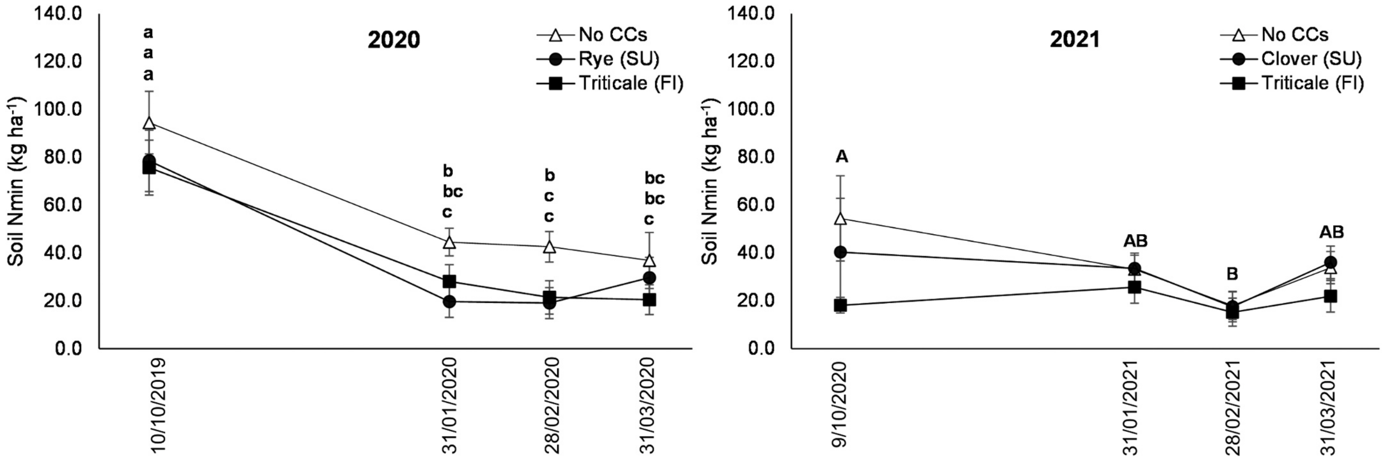

Fig. 4. Soil Nmin (NO$_3$-N) (kg ha^{-1}) in the first 0–40 cm soil layer in 2020 and 2021 (average ± SE with 0.95 confidence interval, reported with the vertical bars). Different lowercase letters indicate significant differences among treatments and sampling date; different uppercase letters indicate 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

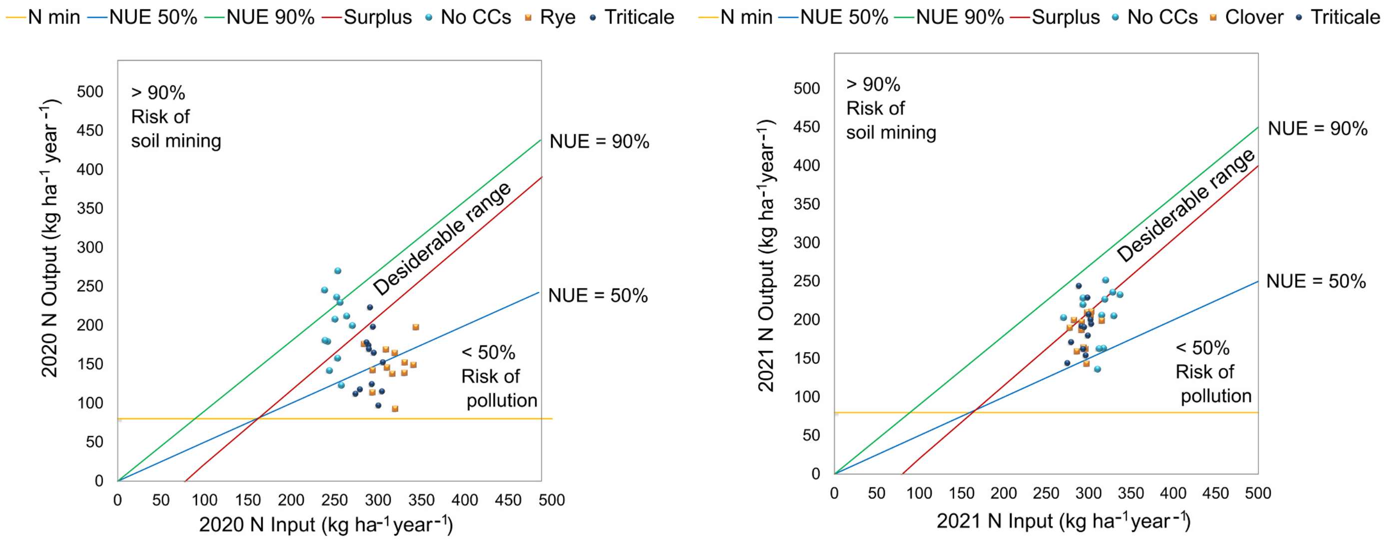

Fig. 5. Graphical presentation of the N use efficiency (NUE) (according to the EU Nitrogen Expert Panel, 2015) in both 2020 and 2021 for each CCs and fallow treatments. The green line corresponds to the NUE = 90%; the red line to the desired maximum surplus (NEU = 80%); the blue line to the NUE = 50%; 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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Chapter 2

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RESEARCH ARTICLE

Satellite imagery and modeling contribute understanding cover crop effect on nitrogen dynamics and water availability

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Abstract
Cover crops (CCs) can affect the cropping systems' N dynamics and soil water content (SWC), but optimizing their potential effects requires knowledge of their growth pattern, N accumulation, and mineralization. For this purpose, a 3-year field experiment was initiated in northeast Italy involving a maize-soybean rotation. The objectives of this study were to (i) evaluate the use of time series vegetation indices (VIs) obtained from the Sentinel-2 satellite for monitoring the growth of CCs and estimating their biomass and N uptake at termination; (ii) investigate the effects of different CCs on cash crop yield and SWC; and (iii) use the simulation model CC-NCALC to predict the nitrogen contribution of CCs to subsequent cash crops. Three CC systems were tested: a fixed treatment with triticale; a 3-year succession of rye, crimson clover, and mustard; and a control with no CCs. Satellite imagery revealed that rye and triticale grew faster during the winter season than clover but slower compared to mustard, which suffered a frost winterkilling. Both grasses and mustard produced greater biomass at termination compared to clover, but none of the CC species affected SWC or yield and N uptake of the cash crop. A net N mineralization of all the CC residues was estimated by the model (except for the N immobilization after triticale roots residues). During the subsequent cash crop season, the estimated clover and mustard N released was around 33%, and the triticale around 3% of their total N uptake, with a release peak 2 months after their termination. The use of remote sensing imagery and a prediction model of CC residue decomposition showed potential to be used as instruments for optimizing the CCs utilization and enhancing cropping water and N fertilization management efficiency; however, it must be further analyzed with other CCs species, environmental conditions, and cropping systems.

Keywords Residue's N release · Soil water content · Mineralization · Remote sensing

1 Introduction

Planting cover crops (CCs) is an acknowledged practice to promote agricultural sustainability by provisioning several agroecosystem services (Wallander et al. 2021) which, among other things, can enhance the cropping systems' fertilization (Dabney et al. 2001; Gabriel et al. 2016) and irrigation management efficiency (Nowak et al. 2022). Nevertheless, enhancing the role of CCs as a suitable solution to reduce chemical fertilizer and water inputs requires a deep knowledge of their growth, nutrient accumulation, and further mineralization (Robertson et al. 2014). Within this frame, the use of satellite images and modeling to monitor the CCs growth and predict their nitrogen (N) contribution to cash crops can be of crucial importance to support farmers and technical advisors to better manage water and N inputs reducing potential environmental impacts and increasing the sustainability of their agricultural systems.

Cover crops performance varies depending on several factors such as planting date and termination, CC species, agricultural management, soil type, elevation, and local and annual climate variability (Poeplau and Don 2015; Lee et al. 2016; Hively et al. 2020). All these factors can affect the CCs' total biomass accumulation, soil coverage, and nutrient uptake, which are directly related to the

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magnitude of agroecosystem services the CCs can provide (Jennewein et al. 2022) both during their growing season and after their termination. For instance, a high winter biomass accumulation is usually related to a high nutrient uptake, which can reduce the soil nutrient concentration potentially leachable (Prabhakara et al. 2015). In addition, the quantity of residues after the CC termination can contribute to the N nutrition of the subsequent crops (Thapa et al. 2018) and affect the soil water content (SWC) available to them (Blanco-Canqui et al. 2020). After termination, CC residues (both incorporated or surface-applied) can release N that might contribute to the cash crop nutrition (Quemada et al. 1997; Cabrera et al. 2005; Thapa et al. 2018, 2022). In addition, their presence during winter can improve soil physical properties (reducing soil bulk density, increasing soil aggregates and water stability, and improving water infiltration and saturated hydraulic conductivity) (Bruce et al. 1991) and increase SWC (Malone et al. 2007). However, on the opposite side, the CC presence can potentially result in nutrients (N) and water competition with the following cash crop, risking impairing the final yield production and quality (Alonso-Ayuso et al. 2014; Gabriel et al. 2014, 2019; Alvarez et al. 2017).

The extent of the N release from the CC residues is strongly affected by multiple factors (residues' quality, management, and environmental conditions) (Poffenbarger et al. 2015; Wagger et al. 1998), and it might even result in N immobilization (Rosolem et al. 2018), in the short period, rather than in an additional N contribution. Indeed, it is still unclear if and to what extent CCs can contribute to the cash crop's N requirements and help reduce the reliance on N fertilizers (Wittwer and van der Heijden 2020). This might vary a lot among CC species, but even in the case of legume CCs (reported to fix more than 100 kg N ha^{-1} $year^{-1}$), it is difficult to predict the actual N quantity available for the subsequent cash crop (Thorup-Kristensen et al., 2003; Büchi et al. 2015).

In this context, it is important to take into consideration that CCs are usually adopted by farmers whose priority is economic success, and they often find the disadvantages of this practice more evident than the advantages (Bergtold et al. 2019). Therefore, the development of reliable tools to monitor the CCs' growth and estimate their possible contribution or immobilization of the N resources might be of utmost importance to evaluate both the advantages and disadvantages related to their adoption in specific sites. This might help the farmers to manage the CC introduction in their agricultural systems maximizing the potential benefit and reducing the potential adverse effects. This is getting even more important after recent global events (including the global pandemic and Ukraine's invasion by Russia) that led to unprecedented N fertilizer price increases threatening the global food and energy supply.

The use of remote sensing techniques to detect and spatialize CCs' growth dynamics and ground cover might be an important tool to investigate the performance of CCs, especially on a large scale, helping the farmers to better manage the CCs according to site-specific needs (Thieme et al. 2020). The retrieval of vegetation indices (VIs), such as the Normalized Difference Vegetation Index (NDVI), from remote sensing imagery is a widespread technique to investigate CCs growth status (Kariyeva and Van Leeuwen 2011; Fan et al. 2020; Gabbrielli et al. 2022). The importance of these remote assessments is gaining increased attention, and it has also been promoted as a first step to defining effective policies that promote the adoption of CCs (Hively et al., 2015; Fan et al. 2020; Nowak et al., 2020). In addition to NDVI, a variety of reflectance-based VIs have been proposed to assess different agronomic variables depending on the regions of the electromagnetic spectrum used. Many of the VIs are based on bands in the visible and near-infrared (NIR) due to the spectral differences between soil and green biomass in these regions caused by the strong chlorophyll absorbance in the visible and the high reflectance of healthy vegetation in the NIR (Daughtry et al. 2000). The VIs based on the red edge region (680–780 nm) enhance the sensitivity to chlorophyll content, and because of the link between chlorophyll and leaf N content, they have the potential to estimate CC N uptake (Yoder and Pettigrew-Crosby 1995; Chen et al. 2010). Also, satellite imagery offers multiple benefits (the capability of acquiring time series images with short revisit time, the wide extension covered by a single image, and the availability of open-access products) that make it suitable for monitoring the performance of CCs in real field conditions (Sishodia et al. 2020).

While the use of remote sensing techniques can be a valid instrument for the CCs' growth monitoring, specific models are required to investigate the CCs' N contribution to the subsequent crop. For this purpose, a web-based model named CC N calculator (CC-NCALC) (developed by the University of Georgia; Woodruff et al. 2018) was created to predict N mineralized or immobilized from decomposing CC residues. The model's main purpose is to help farmers manage N more efficiently, preventing potential problems related to over or under-fertilization. The model, which takes into account many factors affecting the N release from CCs residues (Woodruff et al. 2018; Thapa et al. 2022), has been proposed as a decision support tool to adjust the N fertilizer dose for the cash crops cultivated after CCs seasons.

The present study, conducted in a 3-year maize-soybean rotation in northeast Italy, is aimed at (i) evaluating the efficacy of utilizing time series VIs obtained from the Sentinel-2 satellite for monitoring the growth of CCs, estimating their biomass and N uptake at the termination date; (ii) investigating the various effects of different CCs on cash crop yield and SWC; and (iii) using the simulation model

“Cover Crop N Calculator” (CC-NCALC), which is being used for the first time in Europe, to predict the N contribution of CCs to subsequent cash crops.

2 Materials and methods

2.1 Site description

The study area (6.5 ha) is located at the experimental farm “L. Toniolo” in Legnaro (45° 20′ 53″ N, 11° 57′ 11″ E, 6 m a.s.l.), situated in a plain region of fluvial origin in north-eastern Italy (Padano Valley). The experimental site (Fig. 1) was conventionally managed since 1996 with a non-strict rotation of maize (*Zea maize* L.), wheat (*Triticum aestivum* L.), soybean (*Glycine max* (L.) Merr), sorghum (*Sorghum bicolor* L.), and sugar beet (*Beta vulgaris* L.) (Tolomio and Borin 2019) until October 2019, when the present experiment on winter CCs was instituted up to October 2022. Following the most common rotation system of the study area, maize and soybean were used as cash crops in the rotation with CCs. The soil of the experimental site is classified as Fulvi-calcaric Cambisol (WRBSR 2014). The physical-chemical characteristics (0–40-cm topsoil) are reported in Table 1, whereas the hydrological properties consisted of an upper layer saturated water content (SAT) of 45.8%, drained upper limit (DUL) of 33.9%, and permanent wilting point (PWP) of 13.4%. An impermeable layer at 3-m depth determined a shallow phreatic groundwater table fluctuating from about 0.5–1.5 m in late winter-early spring to 1.5–3 m in summer. More detailed soil hydrological properties for the soil profile down to 2-m depth are reported by Tolomio and Borin (2019).

The area is characterized by a humid subtropical climate (Cfa class in Köppen classification) (Rubel et al. 2017), and it usually has water in excess in autumn and spring and water stress in summer. Weather data (air and soil temperature, precipitation, ET_0) were collected from the meteorological station of the regional agency for environmental protection (ARPAV), located on the “L. Toniolo” farm. Average values for the last 30 years (1992–2022) showed annual rainfall of 830 mm and annual temperature of 13.9 °C with average minimum and maximum temperatures of 8.7 °C and 18.6 °C, respectively. The month with the lowest average minimum temperature is January (− 0.15 °C), while the month with the highest average maximum temperature is July (29.5 °C).

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

Soil characteristics	Values
Sand, 2000–50 μm (%)	36.9 ± 5
Silt, 50–2 μm (%)	44.1 ± 5
Clay, < 2 μm (%)	19.0 ± 2.2
pH	8.0 ± 0.2
Bulk density (Mg m^{-3})	1.62 ± 0.1
Soil organic matter (%)	1.4± 0.1
Inorganic carbon (%)	4.25 ± 0.2
Total Kjeldahl nitrogen (%)	0.09 ± 0.01
NO_3^--N (mg kg^{-1})	576.6 ± 18.1

2.2 Experimental layout and crop management

Three winter CC management strategies were tested in a 3-year crop rotation with silage maize (*Zea mays* L.—in the 1st and 2nd year) and soybean (*Glycine max* L. Merr.—in the 3rd year) as cash crops. The experimental design was a

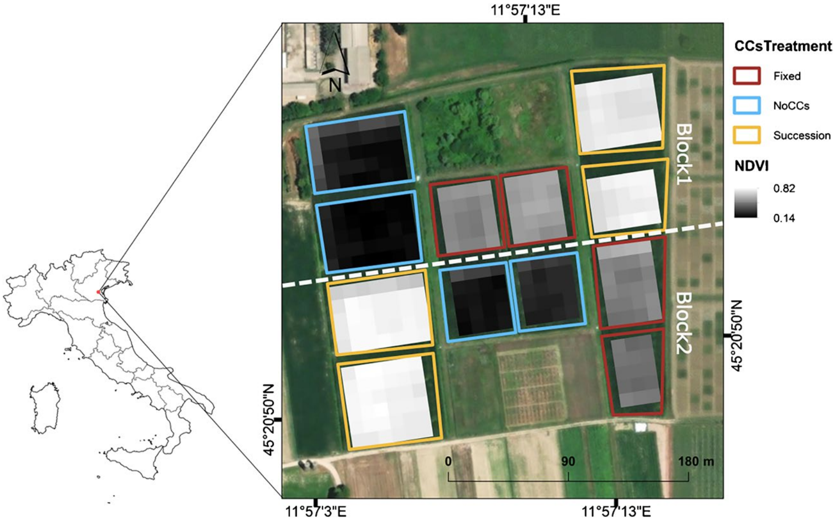


Fig. 1 Map of the experimental site (L. Toniolo farm, Padova, Italy). The experimental design included two blocks with six plots each, where two repetitions of each of the three cover crop treatments were located (Fixed: triticale. Succession: rye in 2020, clover in 2021, mustard in 2022. NoCCs: absence of cover crops). For each plot, the Normalized Difference Vegetation Index (NDVI) was calculated from multispectral satellite images acquired by the Sentinel-2 Level 2A on 31/01/2021. Base map source: Esri, Maxar, Earthstar Geographics.

randomized split-plot including 2 Blocks and 3 CC management strategies in the main plots (Fig. 1). The 3 CC management strategies consisted of (i) a fallow treatment (NoCCs), where the soil was left bare and any chemical or mechanical operation was performed for weed control; (ii) a fixed treatment (Fixed), where triticale (X *triticosecale*) was used every year of the experimentation; and (iii) a succession treatment (Succession), where a 3-year rotation of 3 CC species was tested, including cereal rye in 2020 (*Secale cereale* L.), crimson clover in 2021 (*Trifolium incarnatum* L.), and mustard in 2022 (*Sinapis alba* L.).

The winter CCs were sowed on 10 October 2019, 9 October 2020, and 24 September 2021 with a seeding rate equal to 160 kg ha^{-1} for the grasses, 40 kg ha^{-1} for crimson clover, and 35 kg ha^{-1} for mustard. In all the 3 years, the CCs were terminated on 31 March by shredding with a rotary mulcher for incorporating the residues. Other field operations included subsoil tillage (at 30-cm depth) after the CC termination and rolling harrow for the cash crop seedbed preparation.

The cash crops were irrigated once in 2020 (40 mm on July 12th), 2021 (30 mm on July 12th), and 2022 (40 mm on June 1st). The mineral fertilization consisted of 200 kg N ha^{-1} (16% of urea before sowing and the rest as top-dressing), 80 kg P_2O_5 ha^{-1}, and 80 kg K_2O ha^{-1} before sowing, for maize, and 46 kg P_2O_5 ha^{-1} for soybean. Weeds were controlled, in all the 3 years, with pre-emergence herbicide application at sowing and with post-emergence mechanical control.

2.3 Data collection

2.3.1 Crop sampling

Both CCs and cash crops were sampled for their biomass production at termination and harvest time, respectively. Specifically, three samples of the CCs were collected from each main plot by hand-harvesting the aboveground biomass from 4-m^2 sample areas. The CC roots were sampled from a 0.5 × 0.5 × 0.5 m cube of soil collected in the middle of the aboveground biomass sample area and then washed out from the soil using the method reported by Smit et al. (2013). The cash crops' aboveground biomass was collected at harvest from three sampling areas in each main plot, measuring 18 m^2 for the maize and 13.5 m^2 for the soybean.

All the biomasses were weighed for their fresh weight (FW) and then dried in a thermos-ventilated oven (65 °C) to determine dry matter content. After this, a subsample of each dry biomass was ground to pass a 2-mm sieve and analyzed for carbon (C) (only for CCs) and N contents with a CNS analyzer (CN 802 Carbon Nitrogen Elemental Analyzer, Velp Scientifica, Usmate, Italy).

2.3.2 Soil water content sampling

The soil water content (SWC), sampled in each plot, was measured on a weekly basis (± 4 days) every 10 cm from 0 to 100-cm depth using Sentek's Diviner2000 capacitance sensor (Sentek Environmental Technologies, Kent Town, South Australia). The sampling campaign for the SWC was performed during the CCs growing seasons (from October to March in 2020–2021 and 2021–2022) and the cash crop growing cycle (from June to October 2020, from April to October 2021, and from April to September 2022). The SWC content time-series values were reported for both CCs and cash crop seasons, as the difference between each value measured in each sampling date and the initial ($t = 0$) value measured at the beginning of each growing season (Table 1S).

2.3.3 Sentinel-2 measurements

A time series of multispectral satellite images collected by the Sentinel-2 Level 2A over the experimental site was downloaded from the European Space Agency (ESA) DataHUB server (ESA 2022). For each image, it was visually confirmed that the absence of cloud and cloud shadow over the experimental site. This process resulted in a total of 10 images for each CCs growing season (11 and 26 October, 10 November, 5 and 25 December 2019; 9 and 24 January, 8 and 28 February; 19 March; 10 and 25 October; 24 November 2020; 8 and 14 January, 17 February, 9 and 24 March; 10, 15 and 30 October, 24 November 2021; 8 and 18 January; 2 and 12 February; 9 and 24 March 2022). The Sentinel-2 payload is the Multi-Spectral Instrument (MSI) that measures the radiation reflected from the Earth in 13 spectral bands: four bands at 10-m, six bands at 20-m, and three bands at 60-m spatial resolution (Table 2S). The bands registered reflectance in the visible, red edge and NIR regions at 10- and 20-m spatial resolution were extracted from the pixels that completely lay within each plot. The number of pixels ranges from a minimum of 15 to a maximum of 36 per plot (Fig. 1). The extracted bands of each image were used to calculate various VIs related to different agronomical variables to test their potential for monitoring CC growth and performance (Table 2). This study tested the performance of three VIs based on the visible and NIR (NDVI, GNDVI, and SAVI) and one that incorporates a band from the red-edge region (NDRE) as reported in Table 2. The QGIS software (QGIS Development Team 2020) version 3.28.1. was used to extract the spectral bands. The R software (R Core Team 2021) was used to calculate the VIs of each date.

2.4 CC-NCALC model

The expected N mineralization (or immobilization) from CC residue decomposition was estimated through a web-based

Table 2 The equations and the equations adapted to the multispectral Sentinel-2 bands of the vegetation indices used in this study.

Index	Equation	Equation Sentinel-2	Reference
Normalized Difference Vegetation Index (NDVI)	(R800 – R670)/(R800 + R670)	(B8 – B4)/(B8 + B4)	Rouse et al. 1974
Green NDVI (GNDVI)	(R800 – R550)/(R800 + R550)	(B8 – B3)/(B8 + B3)	Gitelson et al. 1996
Soil-Adjusted Vegetation Index (SAVI)	(1 + 0.5) * (R800 – R670)/(R800 + R670 + 0.5)	(1 + 0.5) * (B8 – B4)/(B8 + B4 + 0.5)	Huete 1988
Normalized Difference Red Edge (NDRE)	(R790 – R720)/(R790 + R720)	(B8 – B6)/(B8 + B6)	Barnes et al. 2000

model called "Cover Crop N Calculator" (CC-NCALC) developed by the University of Georgia (Woodruff et al. 2018; Thapa et al. 2022). The model, described in detail by Woodruff et al. (2018), is a modified and implemented version of the N mineralization and immobilization subroutine from the CERES-N model (Godwin and Allan Jones 1991). It focuses on the decay of the fresh organic matter of the CC residues to calculate the corresponding N accounting and estimate the amount of inorganic N mineralized (or immobilized). The model uses as input data: (i) the dry CC biomass yield and the composition of the CC residues including the fresh matter components (nonstructural carbohydrates, cellulose, and hemicellulose, and lignin) and the C:N ratio; (ii) agricultural management information about the CC sowing and termination time, the management of the residues (incorporated or left on the surface), the type of the agricultural systems (organic or conventional), the cash crop cultivated after the CCs season, and the usual quantity of N fertilizer applied; (iii) environmental parameters such as the daily 0–30 cm SWC and the soil 10 cm temperature for the decomposition period to be simulated, the soil hydrological characteristics (LL, DUL, SAT), the BD, the soil organic carbon content (SOC), and inorganic N.

In the present study (for each of the 3 experimental years), the meteorological data and the soil 10 cm temperature were collected by a weather station located at the experimental site (Vantage Pro Meteo Station by WeatherLink). The 0–30 cm SWC was measured in the experimental field as described in Section 2.3, and the BD and the SOC, as well as inorganic N, as reported in Raimondi et al. (2023). The CCs' biomass C, N, and water contents were determined, for each experimental year, as reported in Section 2.3.1, whereas the fresh organic matter components were estimated using the following equations (R. Thapa, personal communication):

$$\%\text{Carbohydrate} = 24.7 + 10.5 * \%\text{N} \tag{1}$$

$$\%\text{Holo} - \text{cellulose} = 69 - 10.2 * \%\text{N} \tag{2}$$

$$\%\text{Lignin} = 100 - \%\text{Carbohydrate} - \%\text{Holo} - \text{cellulose} \tag{3}$$

The model output gives the cumulative amount of N mineralized (kg N ha^{-1}) (from now on referred to as N release) from the CCs residues over the following cash crop season (140 days in the present study. In addition, it explains how the output N release (kg ha^{1}) estimated by the model can be used to adjust the N fertilization rate.

2.5 Statistical analysis

Descriptive statistics were computed for all the datasets to analyze the main feature of the data distribution. Two analysis procedures were used in the present study to analyze: (1) the cash crops and CCs performances (dry biomass quantity, N uptake, C/N ratio) and the total N released by CCs residues among the different treatments in all 3 years of the experimentation; (2) the SWC during both the CCs and the cash crop season, the VI trend during the CCs growing season, and the N release rate from CC residues (calculated using the slope of lines fitted on monthly N release data) in each year of the experimentation. Marginal and conditional residual distributions were visually checked to detect possible issues of non-normality or heterogeneity of variances, for each analysis performed. The first analysis was performed using linear mixed models ("lmer()" function in R software) (Bates et al. 2015) including the CC treatments and the year as fixed factors and the block as a random effect (Onofri et al. 2016). The second analysis consisted in investigating the temporal trends of the variables using a generalized least squares (GLS) fitting procedure to estimate the standard error accounting for the autocorrelation in the residual series (Cowpertwait and Metcalfe 2009; Campi et al. 2019). Using the gls() function (within the nlme library) on R software, models for repeated measures were built including the CCs treatments as fixed effects. The sampling dates were included in the models as repetition factors, and they were specific for each outcome variable: (i) 2 weeks for the SWC values; (ii) months for VI measurement; and (iii) months for the N release rate by the CCs residues. In addition, simple linear models for repeated measures were also computed for each variable including the same fixed effects. After fitting all the models, the Akaike Information Criterion (AIC) was used to assess and identify the one best fitting the datasets. For all the statistical procedures performed, a Wald test ANOVA of the best-fitting models was used to confirm the sources of variation, and post hoc analyses were carried out with the Sidak test for multiple sets of pairwise comparisons or Tukey test for one set of pairwise comparisons (Lenth et al. 2021). To assess the correlations among the VIs and both CC biomass and N uptake, their Spearman's rank correlation coefficients were computed using the R function "cor" with option method = "spearman." Heatmaps depicting the matrix of Spearman's rank correlation coefficients within each experimental year were then created with R package "gplots." All the statistical analyses were performed with R software (R Core Team 2021).

3 Results

3.1 Meteorological data

For all the experimental years, the yearly rainfall (Fig. 2) was lower than the 30-year average (830 mm) with 701 mm in 2020, 630 mm in 2021, and 464 mm in 2022. The cumulative rainfall registered during the CC season, showed a total of 380 mm in 2020, 279 mm in 2021, and 175 mm in 2022. From October to March, an increase in rainfall events occurred in 2020, while a decreasing pattern was registered in 2021 and 2022. In 2020, the highest cumulative value was registered in March (60 mm), while in 2021 and 2022 the highest precipitation was measured in January and December with 72 and 36 mm, respectively. During the cash crop season, the highest concentration of precipitation events was registered in June and August 2020 (158 mm on average), May 2021 (132 mm), and August and September 2022 (84 mm on average).

The highest and lowest air temperatures were measured in July/August and January for all the experimental years, confirming the pattern observed in the last 30 years. However, the yearly average maximum (25 °C) and minimum (3 °C) temperatures registered during the experimental period were 3.4 and 2.8 times higher than the average 30-year values, respectively. The soil temperature over the 3 years followed the same pattern as the air temperature, with an average maximum value equal to 25 °C in July, and a minimum temperature of 4.6 °C in January. In the summer season (June–August) of both 2020 and 2021, an average soil temperature of 24.3 °C was registered, whereas the air temperature was equal to 23.4 °C (on average). In the summer season of 2022, the soil and air temperatures were equal to 24.1 and 25.5 °C, respectively. During the autumn and winter seasons of all the experimental years, the soil temperature (9.3 °C on average) was slightly higher than the air temperature (8.4 °C). The ET0 distribution showed the lowest values from November to January in all the years of the experimentation, while the highest was between June and July months.

3.2 Cover crops' growing season

3.2.1 Vegetation indices for cover crop monitoring

The temporal evolution of the VIs during the CC season of the 3 experimental years (Fig. 3) showed different patterns depending on the year, the treatment, and their interaction (Table 3S). All the VIs resulted positively correlated with each other's in all experimental years, with Spearman's rho values ranging from 0.73 to 0.99 (Fig. 4). The values of the VIs based on the visible and NIR increased during the first months of all three CCs growing seasons. Differently, the temporal profiles of the VIs in the last months before CC termination differed among years. In 2020 and 2021, the VIs maintain or increase their value until the end of the CCs, whereas, in 2022, the NDVI, GNDVI, and SAVI decreased abruptly in February due to the frost damage suffered by the CCs. Overall, the VIs based on the visible and NIR allowed to discern more the differences among treatments compared to the (NDRE). Particularly, the NDVI displayed the biggest differences between CC treatments, especially when distinguishing between the Fixed and the NoCCs treatment. The identification of the succession treatment varied between years.

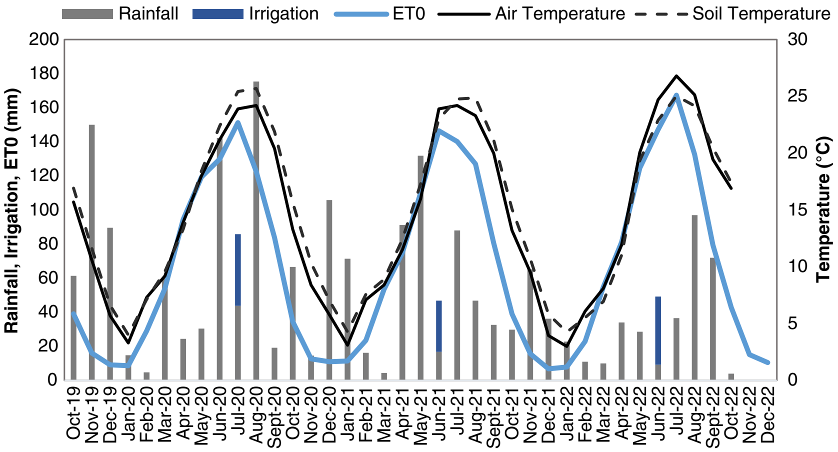


Fig. 2 Monthly rainfall (mm), irrigation (mm), and ET0 (mm) and mean air and soil temperature (°C) during the experimental period at L. Toniolo experimental farm (Padova, Italy).

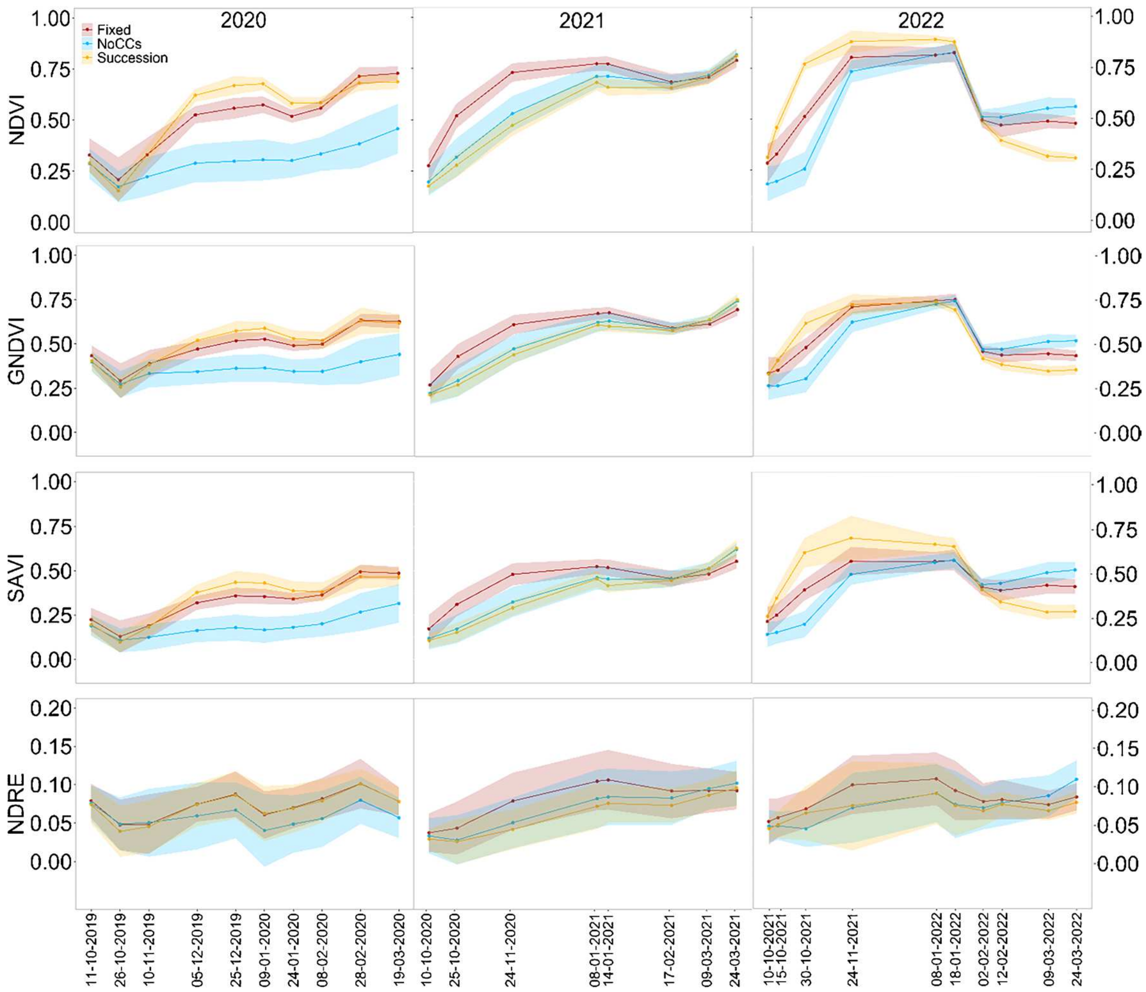


Fig. 3 Temporal evolution of NDVI, GNDVI, SAVI, and NDRE calculated from Sentinel-2 imagery from the experiment for each cover crop treatment (Fixed: triticale. Succession: rye in 2020, clover in 2021, mustard in 2022. NoCCs: absence of cover crops) during the three growing seasons. The points represent the date of image acquisition, the ribbon, and the standard error.

The robustness of the NDVI for CC biomass monitoring was strengthened by the significant relationship with biomass registered at the termination date of the 3 years (Fig. 4). In all cases, Spearman's rho values of NDVI varied between 0.56 and 0.77, even in 2021, when the other VIs' analysis did not correlate with biomass, and only NDVI and GNDVI obtained a moderate correlation. In 2020 and 2022, the NDVI obtained a significant correlation with biomass ($p < 0.01$). A significant correlation between all VIs and N uptake at CC termination was found in 2020, with the highest value obtained by the NDRE (Spearman's rho = 0.71). In 2022, only the NDVI positively correlated with CCs N uptake (Spearman's rho = 0.51).

The highest differences of the VIs based on visible and NIR were observed between both the treatments with CC (Fixed and Succession) and the NoCC treatment in the first experimental year from 5 December 2019 to the termination date (March 19th 2020). The NDVI and SAVI values of both fixed and succession treatments were almost double the NoCC values, while GNDVI values were ≈ 50% higher for both treatments compared to the NoCC treatment. Rye and triticale throughout the first experimental season always showed the same values of all the VIs, except for 3 dates between the end of December and the first of January when rye showed higher NDVI compared to triticale.

In the 2021 growing season, from the end of October to the end of November, all three VIs based on the visible and NIR were significantly higher in the fixed treatment compared to the other two treatments. However, NDVI and SAVI were those displaying the biggest differences between fixed and the other CCs treatments. Higher NDVI values were found for triticale, compared to crimson clover and NoCCs, until the half of February. Any significant difference among treatments was registered in the NDVI from the half of February till the CC termination (end of March). Particularly, triticale NDVI values were 58.8% and 64.9%

higher than both clover and the spontaneous vegetation (No CCs) on October 25th (0.3 on average) and November 24th 2020 (0.5 on average), respectively. While the GNDVI was 40% and 25% higher in the triticale treatment compared to NoCC on October 25th and November 24th. At the end of January 2021, NDVI was equal to 0.7 for triticale and 0.6 for both succession (crimson clover) and NoCCs. From the half of February, the NDVI was equal in all the treatments with average values of 0.6 on February 17th, 0.7 on March 9th, and 0.8 on March 24th 2021. SAVI followed a similar pattern to NDVI but with lower values. This difference between the two indices increased with the growing season as it was < 0.1 at the beginning of October to ≈ 0.2 at the termination date.

Similar to 2021, in the 2022 growing season, the biggest differences between treatments were found in the last two dates of October, with significant differences between Fixed and NoCC treatments in all VIs based on the visible and NIR. However, SAVI and NDVI also displayed significant differences between Succession (mustard) and Fixed (triticale) treatments. In 2022, mustard showed the highest NDVI values until the first days of January, compared to both triticale and NoCCs. On February 2nd 2022, the NDVI values in all the CC treatments showed a significant drop reaching the same value of 0.5 in all the treatments. From this date until the CC termination, triticale and the spontaneous species always showed higher NDVI compared to the mustard. Specifically, on February 12th 2022, the NDVI values were 0.5 for triticale and NoCCs and 0.3 for mustard. On March 9th and 24th 2022, the spontaneous species in the NoCCs showed the highest NDVI values (0.5 and 0.6, respectively), followed by triticale (0.4 on both dates) and mustard (0.3 on both dates). As observed in 2021, SAVI followed a similar pattern to NDVI, and the differences between SAVI and NDVI increased across the growing season, starting from a difference of < 0.1 in October and reaching the biggest difference on January 18th 2022. However, in 2022, after reaching the highest difference, it was reduced to < 0.7 on the termination date on March 24th 2022.

Overall, the VI based on the red-edge region (NDRE) presented an increasing tendency during the growing season of all CC treatments and years but exhibited some reduction of the values in certain winter dates. In 2020, a reduction in NDRE was observed for all CC treatment on December 25th 2019. This reduction was registered 2 weeks before the reduction experienced by the VIs related to green biomass (January 9th 2021) in the triticale and rye treatments (no reduction was observed in the NoCCs treatment in the green biomass VIs). The reduction of NDRE in 2021 started on the same date as the reduction of the VIs related to green biomass (January 14th 2021). However, the reduction in NDRE was only registered by the triticale treatment while the other treatments only showed a decline in the increasing tendency. The reduction in NDRE observed in the 2022 winter dates by all CC treatments was registered 10 days (January 8th 2022) before the decrease observed in the VIs related to green vegetation.

3.2.2 Soil water content

All the SWC values during both the 2020–21 and 2021–22 winter seasons (Fig. 5) showed significant variable trends in time (Table 4S). In the third experimental year, the SWC in both 0–50 and 50–100 layers was also significantly affected by the CC treatment (Table 4S). All the results of post hoc analyses are reported in Tables 4S to 7S. All the SWC values from here on are reported as the positive or negative difference (+ or −) from the initial ($t = 0$) SWC measured at the beginning of each growing season (Table 1S).

In the second and third winter seasons, despite different rainfalls registered (279 mm in 2020–21; 175 mm in 2021–2022), no SWC increase was observed between the CCs sowing and termination time. The winter rainfall allowed to reach field capacity (FC) by January (in both years) in the 0–50-cm depth but not in the deeper soil layer (50–100-cm depth).

Specifically, in the 2020–21 winter season, the highest SWC content values for the first 0–50-cm depth were registered at the end of January and the first days of February. An average of + 34 mm was measured from January 29th to February 3rd 2021, whereas the highest value was registered on February 11th 2021 (+ 39.4 mm). The lowest SWC values were measured both at the beginning and the end of the winter season. On November 12th, the lowest value of − 0.2 mm was registered, while on average + 4.6 and + 22.6 mm were measured from October 28th to November 26th and from March 5th to 25th, respectively. On the deeper soil layer (50–100-cm depth), in 2020–21, after the lowest SWC values (+ 0.19 mm) measured from October 20th to December 4th 2020, an increasing trend was observed until February. In January, higher values (+ 29.3 mm) were registered, before reaching the highest average values of + 47.5 mm in February. Similar values to January were registered in March.

In the 2021–22 winter season, the SWC content in the upper soil layer was significantly higher in the NoCC treatment (+ 37.3 mm on average) compared to the succession (+ 31.6 mm on average), whereas the fixed treatment (35.2 mm on average) did not show any significant difference from the other two. In the 50–100-cm depth, differently, the SWC content measured in both NoCCs and fixed treatments was significantly higher than the succession. Looking at the SWC trend in time for the 0–50-cm layer, it can be observed that the highest average values of + 44.5 mm were measured from the end of December till the end of January. The lowest values were observed at the beginning

Table 3 Total CCs' (aboveground and belowground) dry biomass weight (Mg ha^{-1}) (DB), N content (kg ha^{-1}), C/N ratio, N release accumulation (% of cover crops dry biomass total N uptake—kg ha^{-1}), biomass carbohydrate (%), holocellulose (%), and lignin (%) content of each cover crops treatment at termination time of each year of the experimentation (2020; 2021; 2022) * significance (p value < 0.001); *ns*, not significant (Wald test ANOVA).

Year	Treatment	CC DB (Mg ha^{-1})	CCs' total N uptake (kg ha^{-1})	CCs' total C/N	Total N release (%)	Carbohydrate (%)	Holocellulose (%)	Lignin (%)
2020	NoCCs	1.2e	17.6 d	19.8 de	11.8	39.2 cd	54.8 cd	5.8
	Succession (rye)	7.6 a	90.7 ab	28.1 bc	11.9	37.2 de	56.8 bc	5.9
	Fixed (triticale)	6.6 ab	73.7 abc	28.6 abc	4.5	36.1 ef	57.9 ab	5.9
2021	NoCCs	4.2 cd	80.9 abc	19.2 e	28.4	43.9 ab	50.2 ef	5.7
	Succession (clover)	3.7 d	64.5 c	19.9 de	38.2	45.1 a	49.1 f	5.7
	Fixed (triticale)	5.3 bcd	66.4 bc	27.2 bc	15.6	36.5 def	57.5 abc	5.9
2022	NoCCs	6.9 ab	98.3 a	25.1 cd	15.6	38.3 de	55.7 bc	5.9
	Succession (mustard)	7.5 a	84.1 abc	31.2 ab	28.1	42.1 bc	52.1 de	5.8
	Fixed (triticale)	5.7 abc	62.7 c	34.0 a	-8.8	34.1 f	59.9 a	6.0
Treatment		ns	ns	ns	*	*	*	ns
Time		ns	ns	ns	ns	ns	ns	ns
Treatment × time		*	*	*	ns	*	*	ns

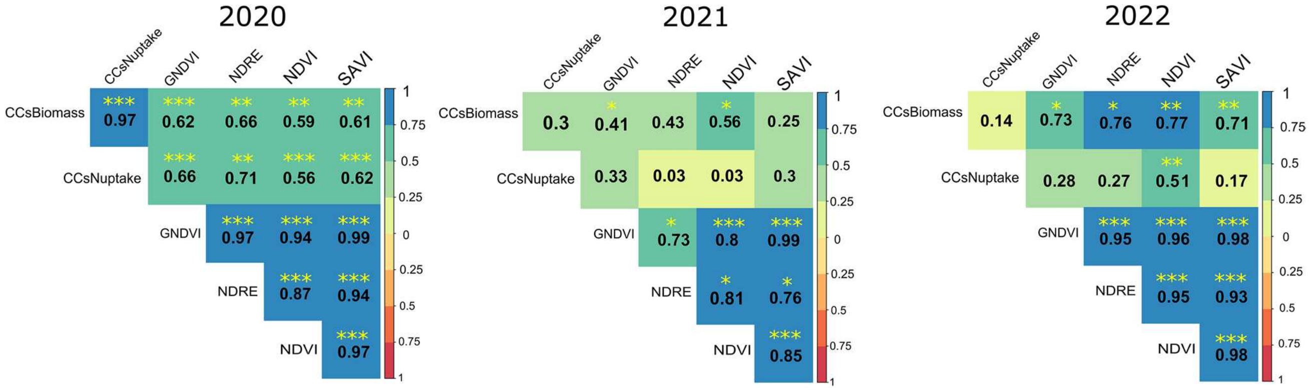


Fig. 4 Spearman correlation heatmap between vegetation indices (GNDVI; NDRE; NDVI; SAVI) and cover crop total (aboveground and roots biomass) biomass production (CCsBiomass) (Mg ha^{-1}) and N uptake (CCsNuptake) (kg ha^{-1}). Statistical differences are marked with * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$). R-values are displayed in different colors, as indicated by the color code on the right side of the heat map.

of the winter season on October 21st (+ 8.26 mm) and at the end of March (+ 25.5 mm). For the 50–100-cm depth, the SWC trend showed the highest values (+ 22.6 mm on average) from the first days of January till the half of March, and the lowest values (+ 4.7 mm on average) only at the beginning of the season from October 21st until December 7th.

The analysis of the SWC at CC termination time showed any significant difference among treatments in both winter seasons (2020–21; 2021–22) except for the values registered in the fixed treatment at the 50–100-cm depth (+ 37.2 mm), higher than both the NoCCs and the succession (18.6 mm).

3.3 Cover crop effect during the cash crop season

3.3.1 Cover crops' residues quality and N release

The CCs' dry biomass quantity and quality (N uptake-kg ha^{-1}, and C/N ratio) (Table 3) in the present study were significantly affected by the interaction between the CC treatment and the year. Both the succession treatments of the first (cereal rye) and last (mustard) years of experimentation showed the highest dry biomass (7.5 Mg ha^{-1} on average), whereas the lowest value was observed in the succession treatment of 2021 (3.7 Mg ha^{-1} for crimson clover). All the

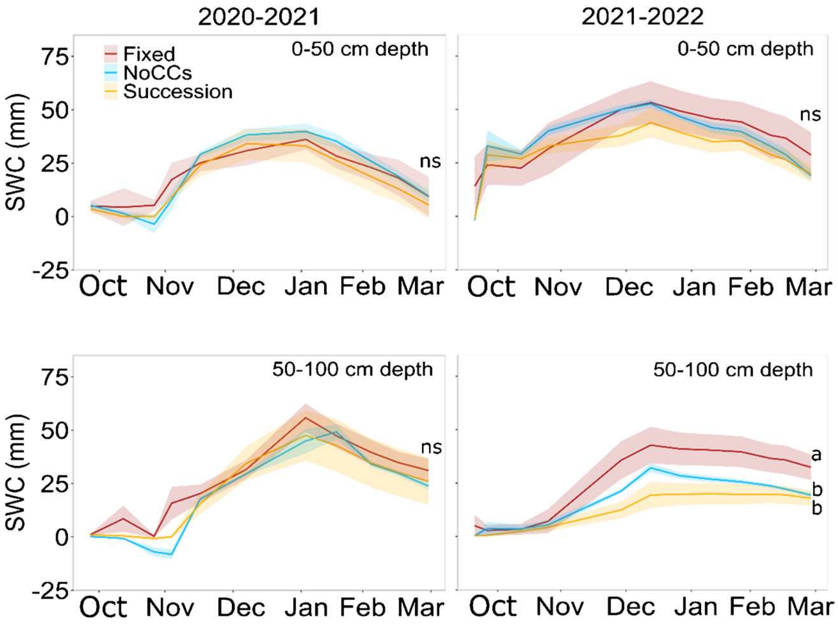


Fig. 5 Average soil water content (SWC) (mm) in the 0–50 and 50–100 cm layers, for the winter season of both 2020–2021 and 2021–2022. Different letters indicate significant differences ($p < 0.01$) among treatments (Fixed: triticale. Succession: rye in 2020, clover in 2021, mustard in 2022. NoCCs: absence of cover crops) in the SWC values measured the last day before the CCs termination (29/03) in both the 2021 and 2022 years. ns: no significant difference among treatments ($p < 0.01$). The standard error is represented by the ribbon around the lines.

other treatments showed intermediate values. The succession and the fixed treatments of 2020 along with the NoCCs of 2021 and 2022 and the succession of 2022 showed the highest total N uptake (85.5 kg ha^{-1} on average) at termination time (March). The fixed treatment of both 2021 and 2022 and the succession treatment of 2021 showed in-between values, while the NoCCs of the first experimental year registered the lowest value (17.6 kg ha^{-1}). Differently, the C/N ratio was higher in the fixed treatment of both the last and the first experimental year and in the succession of 2022 (31.2 on average). The NoCCs of 2020 and 2021 along with the succession treatment of 2021 registered the lowest C/N ratios (19.6 on average), while the rest of the treatments were in-between.

The curves in Fig. 5A, C showed the estimated cumulative daily total N release (kg ha^{-1} day^{-1}) coming from both the aboveground and root biomass residue decomposition over the whole cash crop growing season in 2020, 2021, and 2022. All the total N release curves showed nonstationary trends in time affected by the CCs treatment and by the interaction of the two variables, except for the roots biomass of 2020 which was only affected by the CC treatment (Table 9S).

With regard to the monthly N release rate (kg ha^{-1} month^{-1}) of each treatment in comparison with the others in each experimental year (Fig. 6B, D), it can be noticed that all the N release rates from both the aboveground and root biomass in all the experimental years were affected by the interaction between the time and the CCs treatment, except for the root biomass in 2020 and 2021, that were equal between treatments and constant in time (Table 10S). All the results of the post hoc analyses are reported in Tables 11S to 16S. In 2020, the succession treatment (rye species) showed an increasing pattern from April (0.04 kg ha^{-1} month^{-1}) until June, when it reached the highest N release rate (0.08 kg ha^{-1} month^{-1}). From June, a decreasing rate was registered in the succession treatment until July when it reached the same lower values of both fixed treatment and the NoCCs (0.01 kg ha^{-1} month^{-1}). From August until the cash crop termination, the same lowest N release rate was registered in all the treatments (0.005 kg ha^{-1} month^{-1} on average).

In 2021, the highest N release rate (0.28 kg ha^{-1} month^{-1}) from the aboveground biomass was measured in the NoCCs and the succession treatment (crimson clover) in April and May, followed by the values registered for the same treatments in June (0.13 kg ha^{-1} month^{-1}), in July (0.04 kg ha^{-1} month^{-1}) and at the end of August where the lowest values were registered (0.02 kg ha^{-1} month^{-1}). The N release rate in the fixed treatment instead showed the same values registered in the NoCCs and succession treatment in June, already at the beginning of the season in April, keeping similar values until June, when it reached the lowest values until the end of August. In this month, all treatments registered the lowest N release rate of the season, equal to 0.02 kg ha^{-1} month^{-1}.

The aboveground biomass in 2022 instead showed the highest N release rate in the NoCC treatment in April and May (0.27 kg ha^{-1} month^{-1}) followed by the succession treatment in the same months and the NoCCs in June (0.16 kg ha^{-1} month^{-1}). Starting from June, for the succession treatment, and from July for the NoCC treatments, the lowest N release rates were measured (0.04 kg ha^{-1} months^{-1}) until the end of August. The same lowest value (0.04 kg ha^{-1} month^{-1}) was measured in the fixed treatment from April throughout the entire summer season.

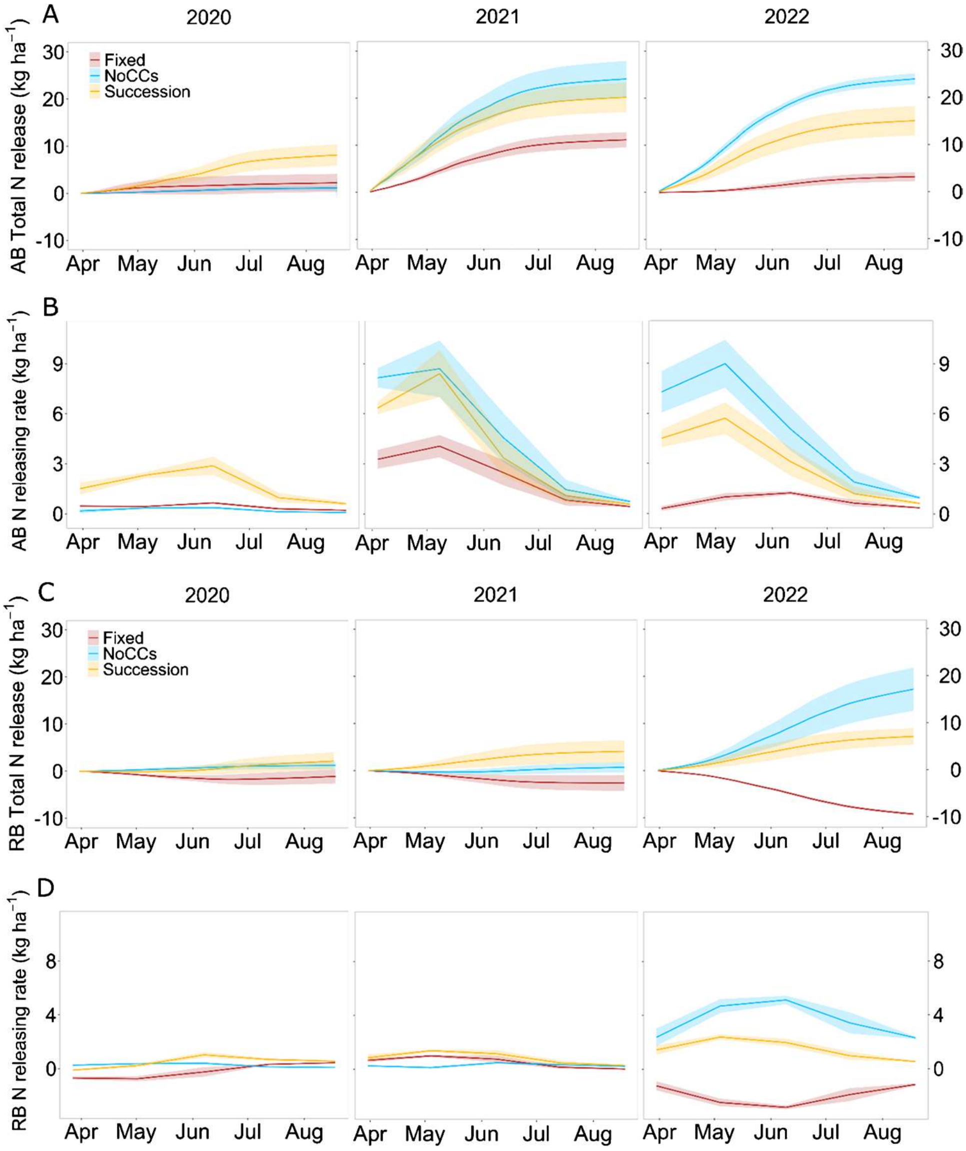


Fig. 6 Daily total N release (kg ha^{-1} day^{-1}) (**A**–**C**) and monthly N release rate (kg ha^{-1} month^{-1}) (**B**, **D**) from both aboveground (AB) and roots (RB) cover crop residues during the summer seasons of each experimental year (2020; 2021; 2022) (Fixed: triticale. Succession: rye in 2020, clover in 2021, mustard in 2022. NoCCs: absence of cover crops). The standard error is represented by the ribbon around the lines.

The N release rate from the roots biomass in 2022, showed the highest value in the NoCC treatment in May and June (0.16 kg ha^{-1} month^{-1}) followed by the values in the NoCCs in April, July, and August, and the succession treatment in May (0.09 kg ha^{-1} month^{-1}). An average value of 0.04 kg ha^{-1} month^{-1} was measured in the succession treatment in April, June, July, and August. In the fixed treatment, the lowest values compared to all the other treatments were registered from April to June (– 0.08 kg ha^{-1} month^{-1}), whereas in July and August, there was a slight increase (– 0.03 kg ha^{-1} month^{-1}) even if the values were still lower than the other two treatments.

The analysis of total cumulative N release during the summer season (Table 3) expressed as a percentage of cover crops dry biomass total N uptake (kg ha^{-1}) showed that it was significantly affected only by the CC treatment. The succession treatment had a higher value (26.1% on average) compared to the fixed (3.7% on average) with the NoCCs showing any significant differences (18.6% on average). The CC residue quality differed for their carbohydrate and holocellulose content percentage according to the CC treatment in interaction with the year, whereas the lignin content did not show any significant difference. The highest percentages of carbohydrates were found in the succession treatment and the NoCCs of 2021, whereas the lowest values were in the fixed treatments of all the years of experimentation (2020, 2021, 2022). The succession treatment and the NoCCs of 2020 and 2022 showed intermediate carbohydrate contents. An opposite result was observed for the holocellulose content where the highest values were registered in the fixed treatment in all three experimental years (2020, 2021, 2022), and the lowest values in both succession and NoCCs treatment in 2021. Intermediate holocellulose contents were observed in the succession treatment of both 2020 and 2022 and the NoCCs 2020.

3.3.2 Soil water content

SWC during the three cash crop seasons (Fig. 7) showed variable trends in time, and only in 2021 and 2022, the SWC in both 0–50 and 50–100 cm was also affected by the CC treatment (Table 17S). The results of all the post hoc analyses are reported in the supplementary materials (Table 18S to 23S). All the SWC values from here on are reported as the positive or negative difference (+ or −) from the initial (*t* = 0) SWC measured at the beginning of each growing season (Table 1S).

In 2020 and 2022 years, the SWC showed low variation from the cash crop sowing to the harvest time. In the first year, around + 27 mm of SWC (0–50 and 50–100 cm depth average of all the treatments) were registered at the beginning of the cash crop season and a total of 435 mm of rainfall were observed over the summer period. In 2022, cumulative rainfalls of 277 mm were measured over the cash crop growing season, and around − 6.3 mm of SWC (0–50 and 50–100 cm depth average of all the treatments) were registered at soybean sowing time (Fig. 7). In 2021, intermediate conditions in terms of rainfall were observed for the summer period (total of 407 mm), and the SWC showed a decreasing pattern in all the treatments from the cash crop sowing date and the harvest time (Fig. 7).

Specifically, in 2020, a similar pattern of SWC values was observed in both 0–50 and 50–100-cm depths. The highest SWC values were registered at the half and end of June (June 12th and 22nd 2020) (+ 31 and + 18 mm for the upper and deeper soil layers, respectively). In July, the SWC content in the upper 0–50 cm reached the lowest values, similar to the end of the season (September 30th and October 7th 2020) (− 0.39 mm), before increasing again in August reaching 13.3 mm. In the deeper soil layer, the lowest SWC values (− 13.3 mm) were reached from the half of July (July 14th 2020) and kept constant till the end of the season (September 30th 2020).

In summer 2021, the SWC in both the soil depths 0–50 and 50–100 cm, resulted significantly higher in the NoCC treatment (− 0.3 and − 3.7 mm in the upper and deeper layers, respectively) compared to the succession treatment (− 5.5 mm in 0–50-cm depth and − 9.1 mm in 50–100-cm depth) which was also higher than the fixed treatment (− 18.4 and − 16.9 mm in the upper and deeper layers).

The SWC values in 2021 were also significantly different over the season in both 0–50 and 50–100-cm depth. In the upper soil layer, the highest SWC values were measured in the half of April and half of May (+ 31 mm), whereas the lowest (− 45.1 mm) were in September. Average values of − 5.7 mm were observed in June and the first half of July, whereas − 26 mm on average were registered at the end of July and the first days of October. In the 50–100-cm depth, the highest value (+ 40.1 mm) was registered on May 26th 2021 followed by the values measured on May 14th and 20th 2021 (+ 17.3 mm). The lowest values were measured from the half of August until the first of October (− 40.4 mm), whereas average values of − 10.9 mm were measured from the end of June until August.

In 2022, the SWC values in both 0–50 and 50–100-cm depths were affected by the different CCs treatment showing higher values in both NoCCs and fixed treatment (− 10.9 mm in the upper soil layer and − 7.5 mm in the deeper soil layer) compared to the succession treatment (− 31 and − 19 mm in the upper and deeper soil layers). During the summer season, the highest values in the upper soil layer were registered on April 28th and June 9th 2022 (− 5.3 mm), while the lowest was on the first days of September (− 40.1 mm). The nonconstant trend during the summer season revealed an average SWC of − 13.2 mm in May, half of June, and July. An average of − 18 mm was registered at the end of June, July, and end of August. In the deeper soil layer, the SWC in 2022 showed the same highest average value of − 6.8 mm from April to the second half of July, followed by an SWC of

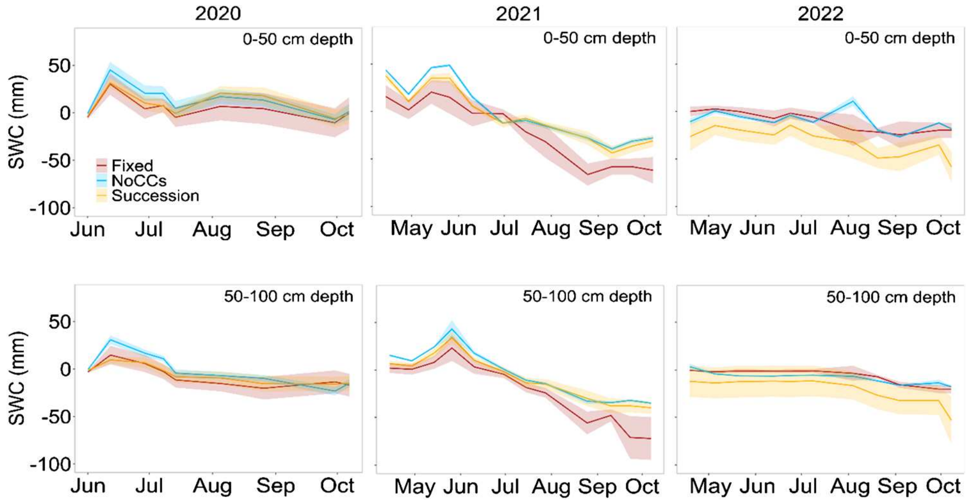


Fig. 7 Average soil water content (SWC) (mm) in 0–50 and 50–100-cm depth for the summer season of 2020, 2021, and 2022. Fixed treatment: triticale; Succession treatment: rye in 2020, clover in 2021, mustard in 2022; NoCCs: absence of cover crops). The standard error is represented by the ribbon around the lines.

− 19.9 mm registered at the end of July and the whole month of August. The lowest value of − 36.8 mm was registered on September 7th 2022.

3.3.3 Cash crop performance

The cumulative marketable dry biomass production over the three experimental years (Fig. 8) did not show any significant difference among the three treatments with an average value of 11.9 Mg ha^{-1}. Similar results were found for the total cumulated N uptake in the cash crop dry marketable biomass (181.2 kg ha^{-1} on average).

4 Discussion

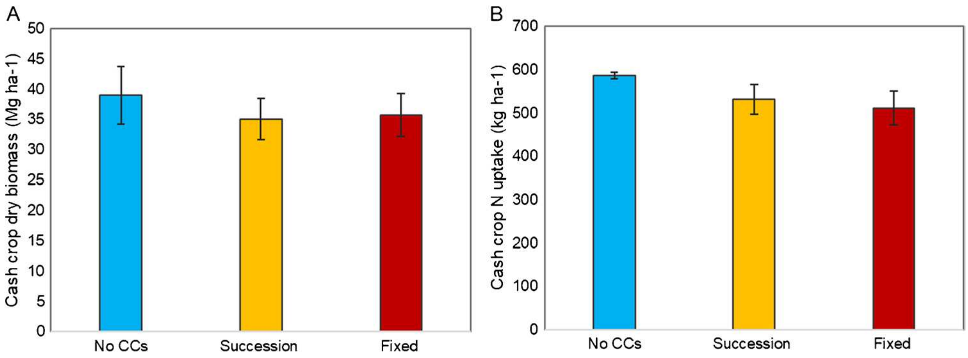
Cover crops growing dynamics can be detected by VIs calculated through satellite images (Kariyeva and Van Leeuwen 2011, Fan et al. 2020). Among the VIs analyzed in this study, the NDVI showed the most robust results for CC monitoring because it was the only vegetation index that presented a significant correlation with CCs biomass all years at the termination date and displayed the most optimal temporal profile, emphasizing differences between CCs treatments. The NDVI has been indeed widely used for assessing vegetation evolution and detecting the percentage of ground cover by the winter CCs (Thieme et al. 2020).

In our study, triticale and rye presented a similar pattern in 2020, both showed rapid growth, reaching an NDVI ≥ 0.5 already in December, confirming previous results showing their potential for removing soil inorganic N and reducing the risk for nitrate leaching (Ruffo and Bollero 2003). However, the faster increase in NDVI, GNDVI, and SAVI values observed for rye suggested a greater biomass production than triticale in December and January, despite the same biomass measured at termination. The result confirmed the rye and triticale biomass production measured in January and in March reported in Raimondi et al. (2023). Similar results were observed in previous studies (Prabhakara et al. 2015; Kim et al. 2017; Vincent-Caboud et al. 2019) reporting rye with greater cold tolerance and faster establishment than triticale in the first months of the winter season. Nevertheless, this trend was not observed in chlorophyll production, as the increase of NDRE displayed similar trends in both rye and triticale. Recently, triticale and barley have been suggested as preferred grasses to be used as CCs compared to rye due to several limitations attributed to the latter (high degree of allelopathy, volunteer cereal rye plants in subsequent phases of the crop rotation, and frequent yield reduction in the subsequent cash crop) (De Bruin et al. 2005; Wells et al. 2016). However, the results of the present study did not show a decrease in maize yield after the rye, and as no differences in SWC after the grasses was observed, there were no symptoms of higher rye preemptive competition. On the other hand, rye was a better N scavenger than triticale in 2020, emphasizing its potential for nitrate leaching control. Therefore, the choice between grass species should be made according to the main ecosystem service expected from the CC in each agricultural system (Ramírez-García et al. 2015).

The triticale confirmed in all the 3 years of the present study a soil coverage pattern attributed to catch crops, with a fast increase in autumn followed by a slight decrease in the colder months, before a second rise starts with the increasing temperature in the early spring. Compared to clover in 2021, triticale showed a faster growth until February, as in a previous study (Hirsh et al. 2021), but the same soil coverage (i.e., NDVI, GNDVI, and SAVI) from the end of February till the CC termination. This result was surprising, considering that crimson clover is not even reported as fast as other clover varieties such as Persian clover (Den Hollander et al. 2007). In our study, the early germination before the cold season might have stimulated a faster clover growth in the mild winter of 2021 (Raimondi et al. 2023) as previously reported for warm-season legumes (Butler et al. 2012). This observation confirms the crucial effect of the establishment and termination dates on CC biomass accumulation in the winter season (Duiker 2014).

Looking at the last winter experimental season, mustard CCs showed a larger and faster soil coverage than triticale (with NDVI = 0.75 and GNDVI and SAVI > 0.6 already at the end of October, 1 month and 1 week after the sowing

Fig. 8 Cumulative dry biomass of cash crops (Mg ha^{-1}) (**A**) and cumulative N uptake (kg N ha^{-1}) by aboveground biomass of cash crops (**B**) in each CC treatment (Fixed: triticale. Succession: rye in 2020, clover in 2021, mustard in 2022. NoCCs: absence of cover crops) for each experimental season (silage maize in 2020 and 2021; soybean in 2022).



date). However, mustard showed a high susceptibility to the frost event that occurred in the first days of February, confirming previous findings of Gabbrielli et al. (2022). Indeed, after the frost event, mustard was the only winter-killed species, compared to grasses and spontaneous vegetation that showed a growth recovery, as already reported by Koudahe et al. (2022). Early winter-killed mustard plants, laid on the soil, were likely to keep controlling soil erosion (Prabhakara et al. 2015), but at the same time might have resulted in N release from residue decomposition. This early N release might have increased surface soil mineral N concentrations (Hirsh et al. 2021) and, at the same time, the risk of N leaching (Dean and Weil 2009). However, the SWC measurement after the frost event showed a decreasing pattern of SWC up until the end of March (CC termination), which suggested a possible growth activity of the smaller mustard plants that managed to recover after the frost events (being protected by the higher plants). A photographic survey of plants on February 2022 (data not shown) confirmed the presence of small mustard plants growing among the dead residues of the winter-killed plants. These results suggest that N released from the winter-killed mustard residue decomposition might have not been leached but remained potentially available for the following cash crop (Snapp et al. 2005; White et al. 2017).

Despite all the VIs based on the visible and NIR, NDVI identified better the CC growing dynamics compared to NDRE, but NDRE was able to detect the drop of chlorophyll content suffered by the winter-killed plants up to 10 days before the other VIs. This is attributed to the highest sensitivity of the red edge bands to chlorophyll content than the visible bands (Xie et al. 2019). This is also supported by the fact that the NDRE obtained the highest correlation with N uptake in 2020. Different studies demonstrated that the sensitivity of the red edge region to chlorophyll content and N uptake increase with hyperspectral sensors capturing narrow bands of < 5 nm spectral resolution (Berger et al. 2020; Raya-Sereno et al. 2021), as compared to the 15-nm spectral resolution of the Sentinel-2 B6. For this reason, the upcoming satellite missions such as Landsat Next or Copernicus hyperspectral imaging mission for the environment (CHIME) that will provide open-access products with global hyperspectral measurements are expected to provide improvements in the CC dynamics monitoring.

In this study, the VIs analyzed showed good performance in monitoring CCs' growth and parameters. However, due to the empirical basis of the VIs, the reliability of this approach should be tested with different CC species, soils, and climate conditions in order to provide accurate CC management recommendations. This is especially important when using VIs because they are based on the relationship between few spectral bands and therefore ignore information from other wavelengths collected by the sensor, which can lead to a lack of transferability (Camino et al. 2022). For this reason, it is important to test alternative modeling approaches for CC monitoring that rely on the entire spectra, instead of considering only VIs. Modeling approaches such as multiple endmember spectral mixture analyses (MESMA; Roberts et al. 1998) or radiative transfer models like PROSAIL (Jacquemoud et al. 2009) can be an alternative to VIs to enhance CC monitoring with remote sensing techniques and, therefore, should be investigated in future research. The MESMA approach performs fractional cover maps based on pure spectra of the different land cover classes, which can be collected from the same image (Meerdink et al. 2019). MESMA has demonstrated success in detecting agricultural management practices (Shivers et al. 2019) and fractional covers (Dennison et al. 2019) using time series acquisition, but its applicability for CC monitoring remains untested. On the other hand, PROSAIL applied to Sentinel-2 imagery has shown satisfactory results in N uptake monitoring (Bossung et al. 2022). However, the application of PROSAIL specifically for CCs monitoring has only been explored by Wang et al. (2023), who reported promising results in estimating aboveground biomass and N uptake using airborne hyperspectral sensors. Therefore, future research should delve into the potential of these advanced techniques for comprehensive CCs monitoring and management.

The introduction of winter CCs is reported as a valid agronomic practice to improve soil physical properties as well as increase SWC (Malone et al. 2007). In the critical periods of our study (CC termination and before the cash crop sowing in 2021 and 2022), all treatments showed the same SWC in the upper 0–50-cm depth. By the end of winter, after the colder months when the plants' transpiration and the water uptake were at a minimum, the CCs showed a growth resumption with the increasing temperature (both air and soil) and related soil nitrification activity. In this period, there was water consumption by CCs, but it did not impair the SWC available by the time of cash crop sowing, compared to the spontaneous vegetation in the NoCC treatment, confirming previous findings (Alonso-Ayuso et al. 2018a, b). The CCs and the spontaneous species indeed did not make the SWC during summer a limiting factor for maize and soybean final yield production and quality. In 2021 indeed, the maize after both the CC species (triticale and crimson clover) had lower SWC available compared to the NoCC treatment from the end of June until August when maize water demand is usually critical for optimizing yield (NeSmith and Ritchie 1992). In 2022, a similar pattern was observed for the soybean at the beginning of the reproductive stage, which had lower SWC availability after mustard CCs compared to both triticale and NoCC treatments. The low rainfall registered in both the 2020 and 2021 summer seasons might have resulted in the SWC not being sufficient to replenish soil water levels after CC termination; in addition, the incorporation of the CC residues may not have reduced soil evaporation as much as it is usually reported for residues left on the surface (Dabney 1998; Unger and Vigil 1998). Moreover, CC effect on soil physical property improvement, which can increase infiltration and enhance water storage capacity is usually reported after long-term use of CCs (Steele et al. 2012; García-González et al. 2018; Çerçioğlu et al. 2019), whereas the present study is a 3-year-long experiment. Overall, pre-emptive water competition caused by CCs is the result of multiple factors, and despite the changes in SWC registered during the three seasons, no significant effect on cash crop biomass was observed.

Looking at the CCs' effect on the N dynamics during the whole experimental period, it has been observed that the cumulative final N uptake of the cash crops was the same in the CC and NoCC treatments. Although the model estimated a higher cumulative total quantity of N released from the CC residues in the succession treatment compared to the NoCCs and fixed treatments, it did not affect the final cash crop N uptake. This was likely due to the low amount of N released registered on average in all the treatments, which averaged about 16.3% of the total N uptake of the CCs. This result is related to the total quantity of CC biomass produced in the present study at the termination time (end of March), which was in some cases lower compared to average values registered in other studies conducted in sub-humid regions (Lu et al. 2000; Prieto and Ernst 2012; Ruis et al. 2019). The analysis of the N-release rate estimated by the model in each year of the experimentation allowed to show different patterns in the CC treatments in the 3 years. The simulation showed a net N mineralization from all the CCs and the spontaneous vegetation aboveground biomass residues (in all the experimental years), whereas a net N immobilization from the triticale roots biomass. The N mineralization of the CCs residues showed the same pattern reported in previous studies about CCs incorporated residues (Kuo and Sainju 1998; Lawson et al. 2013; Poffenbarger et al. 2015) with the highest N release rate reached around 2 months after the CC termination, followed by a decreasing rate until the cash crop harvest. A similar pattern was observed for the triticale root residue immobilization, which was highest 2.5 months after the CC termination. Even though it is usually reported that all the triticale biomass residues lead to N immobilization (Rosolem et al. 2018; White et al. 2016), in our study, it was registered only for the root biomass.

The N release pattern, from the CC residues, showed a possible fit with the N requirement pattern of summer cash crops (Raimondi et al. 2021) such as maize (highest N uptake around 30–40 days after planting). It is indeed reported that introducing CCs may result in better synchrony of N mineralization with the N uptake by the subsequent crop (Lara Cabezas et al. 2004). However, the cumulative N released by the CCs in our study was lower than the cash crop N demand. This result suggests that the pattern of the N release rate from all the CC residues (legumes, brassicas, and grasses), in addition to the amount of N released, should be further investigated in long-term experiments to evaluate their possible contribution to more efficient and sustainable N fertilization.

The N release estimated in the present study also confirmed the potential impact of the environmental conditions on the N mineralization-immobilization processes. Regardless of the CC species, in both the 2020 and 2021 years, increasing trends of the estimated N release rates (after the CCs termination) were estimated in correspondence to both increasing SWC and soil temperature values in rye, clover, and NoCC treatments. These observations confirmed previous results (Torres et al. 2015; Fraser and Hockin 2013; Bontti et al. 2009) showing the crucial impact of SWC and temperature on residue decomposition rates, especially in the early stages after their termination (Soong and Nielsen 2016). Soil moisture and temperature conditions might have also affected the soil micro-fauna, which in turn might have fostered the decomposition rates by increasing detritus surface area through fragmentation and fostering greater microbial colonization (Londoño-R et al. 2013). The CC residue management through incorporation, instead, might have affected less the residue moderation of soil temperature and water content compared to what was observed in the cases of surface application of CC residues (Cook et al. 2010).

The N dynamics, observed over the 3 years, revealed different impacts of CC species and spontaneous vegetation on the N cycle. Interestingly, in the present study, the spontaneous species (starting from the second year of experimentation) showed the same winter soil cover as CCs, and a similar N released quantity than clover (likely due to the high presence of spontaneous clover within the weeds species composition), higher than triticale, during the cash crop season. While the potential of clover as green manure was widely reported (Coombs et al. 2017; Yang et al. 2019), the results of our study demonstrated that further investigations into the potential role of spontaneous vegetation in the N dynamics of conventional agricultural systems might be worthwhile (Li et al. 2020). The results observed for the mustard allowed to confirm previous findings reporting Brassicaceae species as intermediate species between grasses and legumes. It is indeed reported that they can accumulate similar N to grasses in the winter period but decompose faster (thanks to their C:N ratio) supplying substantial plant nutrition similar to legumes (Collins et al. 2007; Finney et al. 2016). Looking at the two grasses species in 2020, rye species showed a higher total N release compared to triticale, despite the same SWC and soil temperature measured in the two treatments. As reported by Thapa et al. (2021, 2022), rye species might show high potential in terms of N release (even with surface-applied residues), especially when terminated at tillering stage.

In summary, the results of the present study suggested that different CC species might be preferred according to the subsequent cash crops in the agricultural rotation, the environmental conditions of each specific site under analysis, and the main purposes for the CCs introduction. Leguminous species, such as clover, can be considered and evaluated if the main objective is to optimize the N fertilization and reduce fertilization dose. Further study should be conducted to evaluate the CC effect with sub-optimal N fertilization doses. Mustard was revealed as a potential candidate for winter N leaching control and for optimizing cash crop N fertilization. It is highly susceptible to winter climatic conditions though, risking being winter-killed and precociously degraded. Rye and triticale, as grass species, confirmed their validity as catch crops for the winter period. However, long-term experiments with both grasses are needed to better assess their effect on the N nutrition dynamics.

5 Conclusions

Optimizing the cover crops' potential benefits on the N dynamics and the soil water content of agricultural systems requires a deep understanding of their growth pattern, N accumulation, and subsequent mineralization.

The use of remote sensing tools, such as satellite images (from which derive VIs) in the present study allowed to reliably monitor the CCs' growing pattern and underlined the site-specific differences among CC species' soil coverage during the winter season. Despite different developments, all the CC species and the spontaneous vegetation in the control treatment used the soil water and N resources for their growth without competing with the subsequent cash crops. The introduction of CCs in the present study did not indeed affect the cash crops yield production and quality.

Nevertheless, the estimation of the CC residue decomposition through a web-based model (CC-NCALC) revealed that CCs in the present study can differently affect the soil N dynamics enhancing N mineralization and N immobilization after incorporation of CC residues of clover and grasses, respectively. The use of the prediction model allowed to estimate the CC N contribution to the subsequent crop. Despite the specific results of the present study (little N contribution estimated from all the CCs likely due to the low CCs biomass production), the application of the model is able to provide information potentially helpful to increase the management efficiency of cash crop N fertilization.

Both the use of prediction model for CC residues' N release and remote sensing tools can be valid instruments to optimize the CC utilization enhancing crop water and the N fertilization management efficiency.

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Authors' contributions M. Quemada and M. Borin initiated and conceived the study. C. Maucieri and G. Raimondi collected the field data. J. L. Pancorbo and G. Raimondi collected the satellite images and derived the vegetation indices. C. Maucieri and G. Raimondi compiled the datasets and designed the data analysis. M. Cabrera compiled the dataset and ran the CC-NCALC model runs. G. Raimondi carried out the statistical analysis. The first draft of the manuscript was written by G. Raimondi, and all the authors commented on and revised previous versions of the manuscript. All the authors have read and approved the final manuscript.

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Data availability The data that support the findings of this study are available on request from the corresponding author.

Code availability The R-scripts for the statistical analyses generated during the current study are available on request from the corresponding author.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

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Supplementary

Table 1S. Initial average SWC (mm) (t=0) ± standard deviation, measured at both cash crops and CCs sowing date over the 3 years of experimentation, in each cover crops treatment (Fixed: triticale. Succession: rye in 2020, clover in 2021, mustard in 2022. NoCCs: absence of cover crops).

Seasons	Year	CCs treatment	Soil depth (cm)	Initial SWC (t=0)
Winter	2020-2021	NoCCs Succession Fixed	0-50	85.9 ± 10.6 88.9 ± 23.6 106.4 ± 16.8
		NoCCs Succession Fixed	50-100	186.8 ± 10.3 188.3 ± 73.2 229.2 ± 24.4
	2021-2022	NoCCs Succession Fixed	0-50	60.1 ± 4.4 57.5 ± 17.2 80.5 ± 10.0
		NoCCs Succession Fixed	50-100	153.1 ± 12.7 154.3 ± 44.8 189 ± 35.7
Summer	2020-2021	NoCCs Succession Fixed	0-50	79.3 ± 14.9 85.7 ± 29.1 106.9 ± 18.5
		NoCCs Succession Fixed	50-100	217.3 ± 25.8 218.4 ± 90.7 263.1 ± 48.3
	2021-2022	NoCCs Succession Fixed	0-50	89.2 ± 9.9 90.9 ± 25.2 114 ± 10.1
		NoCCs Succession Fixed	50-100	224.4 ± 15.9 234.3 ± 65.7 284.1 ± 29.2
	2022-2023	NoCCs Succession	0-50	83.1 ± 9.2 98.7 ± 11.1

		Fixed		94.1 ± 23.2
		NoCCs	50-100	192.7 ± 11.1
		Succession		212.3 ± 29.6
		Fixed		219.6 ± 32.3

Table 2S. Spectral and spatial resolution of the Sentinel-2 bands.

Sentinel-2			
Name	**Center (nm)**	**Spectral resolution (nm)**	**Spatial resolution (m)**
B1	443	20	60
B2	490	65	10
B3	560	35	10
B4	665	30	10
B5	705	15	20
B6	740	15	20
B7	783	20	20
B8	842	115	10
B8A	865	20	20
B9	940	20	60
B10	1375	30	60
B11	1610	90	20
B12	2190	180	20

Table 3S. Vegetation indices' sources of variation for each experimental year and each cover crops treatment (Fixed: triticale. Succession: rye in 2020, clover in 2021, mustard in 2022. NoCCs: absence of cover crops). * Significance; ns = not significant (Wald test ANOVA).

Years	**Source of variation**	**p value***			
		NDVI	GNDVI	NDRE	SAVI
CCs season 2020	Treatment	<0.001	<0.001	<0.001	<0.001
	Time	<0.001	<0.001	<0.001	<0.001
	Treatment X Time	<0.001	<0.001	<0.001	<0.001
CCs season 2021	Treatment	<0.001	<0.001	<0.001	<0.001

	Time	<0.001	<0.001	<0.001	<0.001
	Treatment X Time	<0.001	<0.001	<0.001	<0.001
CCs season 2022	Treatment	<0.001	<0.001	<0.001	<0.001
	Time	<0.001	<0.001	<0.001	<0.001
	Treatment X Time	ns	<0.001	<0.001	<0.001

Table 4S. Soil water content's sources of variation for each year and cover crops treatment (Fixed: triticale. Succession: rye in 2020, clover in 2021, mustard in 2022. NoCCs: absence of cover crops) * Significance; ns = not significant (Wald test ANOVA).

Seasons	**Year**	**Soil depth (cm)**	**Source of variation**	**p value ***
CCs season	2020-21	0-50	Treatment	ns
			Time	<0.001
			Treatment*Time	ns
		50-100	Treatment	ns
			Time	<0.001
			Treatment*Time	ns
	2021-2022	0-50	Treatment	<0.005
			Time	<0.001
			Treatment*Time	ns
		50-100	Treatment	<0.01
			Time	<0.001
			Treatment*Time	ns

Table 5S. Soil water content average values (emmeans) at 0-50 cm depth during cover crops seasons of 2020-2021. Standard Error (SE); upper and lower confidence interval (CI) (at 0.95 confidence interval). Different letters indicate significant differences (p<0.05) between the levels of the source of variation.

Data	**emmean**	**SE**	**lower.CI**	**upper.CI**	**Significance (p<0.05)**
2020-10-20	4.68734844	3.61042505	-6.49530294	15.8699998	fgh
2020-10-28	2.212276	3.61042505	-8.97037538	13.3949274	gh
2020-11-05	3.23837278	3.61042505	-7.9442786	14.4210242	fgh
2020-11-12	0.29769808	3.42514995	-10.3110965	10.9064927	h
2020-11-18	3.41229117	3.42514995	-7.19650343	14.0210858	gh
2020-11-26	5.35888025	3.42514995	-5.24991434	15.9676748	fgh
2020-12-04	13.7767378	3.42514995	3.16794316	24.3855323	defgh

2020-12-11	30.6455214	3.42514995	20.0367268	41.254316	abcd
2020-12-17	27.9477714	3.42514995	17.3389768	38.556566	abcde
2020-12-21	25.9375131	3.42514995	15.3287185	36.5463077	abcde
2021-01-07	33.1415214	3.42514995	22.5327268	43.750316	abc
2021-01-16	26.7351881	3.42514995	16.1263935	37.3439827	abcde
2021-01-20	26.9207714	3.42514995	16.3119768	37.529566	abcde
2021-01-29	33.1210214	3.42514995	22.5122268	43.729816	abc
2021-02-03	36.0244381	3.42514995	25.4156435	46.6332327	ab
2021-02-11	39.4132714	3.42514995	28.8044768	50.022066	a
2021-02-17	29.5726881	3.42514995	18.9638935	40.1814827	abcd
2021-02-25	26.9527714	3.42514995	16.3439768	37.561566	abcde
2021-03-05	21.8138548	3.42514995	11.2050602	32.4226493	abcdef
2021-03-10	19.8830214	3.42514995	9.27422682	30.491816	bcdefg
2021-03-17	16.0567714	3.42514995	5.44797682	26.665566	cdefgh
2021-03-25	11.1043781	3.42514995	0.49558349	21.7131727	efgh
2021-03-31	4.57051542	3.42514995	-6.03827918	15.17931	fgh

Table 6S. Soil water content average values (emmeans) at 50-100 cm depth during cover crops seasons of 2020-2021. Standard Error (SE); upper and lower confidence interval (CI) (at 0.95 confidence interval). Different letters indicate significant differences ($p<0.05$) between the levels of the source of variation.

Data	**emmean**	**SE**	**lower.CI**	**upper.CI**	**Significance (p<0.05)**
2020-10-20	-0.0045222	3.09535272	-9.59182835	9.58278396	j
2020-10-28	-2.67725579	3.09535272	-12.2645619	6.91005037	j
2020-11-05	3.26793185	3.09535272	-6.3193743	12.855238	hij
2020-11-12	0.41517174	2.93650942	-8.68014549	9.51048896	j
2020-11-18	0.5308875	2.93650942	-8.56442972	9.62620472	j
2020-11-26	-0.53628049	2.93650942	-9.63159771	8.55903674	j
2020-12-04	2.7563584	2.93650942	-6.33895882	11.8516756	ij
2020-12-11	18.9222399	2.93650942	9.82692271	28.0175572	fgh

2020-12-17	18.0022399	2.93650942	8.90692271	27.0975572	ghi
2020-12-21	19.1794622	2.93650942	10.0841449	28.2747794	fgh
2021-01-07	32.0370316	2.93650942	22.9417144	41.1323488	cdefg
2021-01-16	27.4256427	2.93650942	18.3303255	36.5209599	defg
2021-01-20	26.6177955	2.93650942	17.5224783	35.7131127	defg
2021-01-29	35.5657122	2.93650942	26.4703949	44.6610294	cde
2021-02-03	51.3891844	2.93650942	42.2938672	60.4845016	ab
2021-02-11	52.6923788	2.93650942	43.5970616	61.787696	a
2021-02-17	46.1698788	2.93650942	37.0745616	55.265196	abc
2021-02-25	41.6205733	2.93650942	32.525256	50.7158905	abcd
2021-03-05	36.0134205	2.93650942	26.9181033	45.1087377	bcde
2021-03-10	34.0621705	2.93650942	24.9668533	43.1574877	cdef
2021-03-17	31.3118927	2.93650942	22.2165755	40.4072099	cdefg
2021-03-25	28.4318233	2.93650942	19.336506	37.5271405	defg
2021-03-31	22.9437504	2.93650942	13.8484332	32.0390676	efg

Table 7S. Soil water content average values (emmeans) at 0-50 cm depth during cover crops seasons of 2021-2022. Standard Error (SE); upper and lower confidence interval (CI) (at 0.95 confidence interval). Different letters indicate significant differences ($p<0.05$) between the levels of the source of variation.

Data	**emmean**	**SE**	**lower.CI**	**upper.CI**	**Significance (p<0.05)**
2021-02-11	39.4132714	3.42514995	28.8044768	50.022066	a
2021-02-03	36.0244381	3.42514995	25.4156435	46.6332327	ab
2021-01-07	33.1415214	3.42514995	22.5327268	43.750316	abc
2021-01-29	33.1210214	3.42514995	22.5122268	43.729816	abc
2020-12-11	30.6455214	3.42514995	20.0367268	41.254316	abcd
2021-02-17	29.5726881	3.42514995	18.9638935	40.1814827	abcd
2020-12-17	27.9477714	3.42514995	17.3389768	38.556566	abcde
2021-02-25	26.9527714	3.42514995	16.3439768	37.561566	abcde
2021-01-20	26.9207714	3.42514995	16.3119768	37.529566	abcde
2021-01-16	26.7351881	3.42514995	16.1263935	37.3439827	abcde
2020-12-21	25.9375131	3.42514995	15.3287185	36.5463077	abcde
2021-03-05	21.8138548	3.42514995	11.2050602	32.4226493	abcdef
2021-03-10	19.8830214	3.42514995	9.27422682	30.491816	bcdefg
2021-03-17	16.0567714	3.42514995	5.44797682	26.665566	cdefgh
2020-12-04	13.7767378	3.42514995	3.16794316	24.3855323	defgh
2021-03-25	11.1043781	3.42514995	0.49558349	21.7131727	efgh
2020-11-26	5.35888025	3.42514995	-5.24991434	15.9676748	fgh
2020-10-20	4.68734844	3.61042505	-6.49530294	15.8699998	fgh
2021-03-31	4.57051542	3.42514995	-6.03827918	15.17931	fgh
2020-11-18	3.41229117	3.42514995	-7.19650343	14.0210858	gh
2020-11-05	3.23837278	3.61042505	-7.9442786	14.4210242	fgh
2020-10-28	2.212276	3.61042505	-8.97037538	13.3949274	gh
2020-11-12	0.29769808	3.42514995	-10.3110965	10.9064927	h

Table 8S. Soil water content average values (emmeans) at 50-100 cm depth during cover crops seasons of 2021-2022. Standard Error (SE); upper and lower confidence interval (CI) (at 0.95 confidence interval). Different letters indicate significant differences (p<0.05) between the levels of the source of variation.

Data	**emmean**	**SE**	**lower.CI**	**upper.CI**	**Significance (p<0.05)**
2022-01-12	26.2851278	1.67891143	21.0859635	31.4842922	a
2022-02-02	24.8189716	1.67891143	19.6198072	30.018136	ab
2022-01-20	24.6343778	1.67891143	19.4352134	29.8335422	ab
2022-01-26	24.3993507	1.67891143	19.2001863	29.5985151	ab
2022-02-10	23.7719217	1.67891143	18.5727573	28.971086	ab
2022-02-16	22.9789227	1.67891143	17.7797583	28.1780871	ab
2022-02-24	22.9761452	1.67891143	17.7769808	28.1753096	ab
2022-03-03	22.5760638	1.67891143	17.3768994	27.7752281	ab
2022-03-10	21.1238657	1.67891143	15.9247013	26.3230301	ab
2022-01-05	21.0098339	1.67891143	15.8106695	26.2089983	ab
2022-03-17	20.3190539	1.67891143	15.1198895	25.5182183	ab
2022-03-23	19.1816105	1.67891143	13.9824461	24.3807749	abc
2021-12-29	17.6167988	1.67891143	12.4176344	22.8159632	abc
2022-03-29	17.412361	1.67891143	12.2131967	22.6115254	abc
2021-12-22	16.7092072	1.67891143	11.5100428	21.9083716	bc
2021-12-15	16.2957915	1.67891143	11.0966271	21.4949558	bc
2021-12-07	10.565179	1.67891143	5.36601458	15.7643433	cd
2021-11-25	7.20870868	1.67891143	2.0095443	12.4078731	d
2021-11-18	4.49028549	1.67891143	-0.70887889	9.68944986	d
2021-11-12	3.88280521	1.67891143	-1.31635917	9.08196958	d
2021-10-21	3.5570091	1.67891143	-1.64215528	8.75617347	d
2021-11-04	3.53145757	1.67891143	-1.66770681	8.73062195	d
2021-10-27	3.13862507	1.67891143	-2.06053931	8.33778945	d

Table 9S. Sources of variation for the total N release (kg ha^{-1}) in each experimental season (2020; 2021; 2022). * Significance; ns = not significant (Wald test ANOVA).

Year	**CCs biomass**	**N release Source of variation (p values*)**		
		Treatment	Time	Treatment x Time
2020	Aboveground	<0.05	<0.001	<0.01
	Roots	<0.001	ns	ns
2021	Aboveground	<0.001	<0.01	<0.01
	Roots	<0.001	ns	<0.001
2022	Aboveground	<0.001	<0.01	<0.01
	Roots	<0.001	<0.01	<0.01

Table 10S. Sources of variation for the monthly N release rate (kg ha^{-1} month^{-1}) in each experimental season (2020; 2021; 2022). * Significance; ns = not significant (Wald test ANOVA).

Year	**CCs biomass**	**N release rate Source of variation (p value*)**		
		Treatment	Time	Treatment X Time
2020	Aboveground	<0.001	<0.001	<0.001
	Roots	ns	ns	ns
2021	Aboveground	<0.001	<0.001	<0.01
	Roots	ns	ns	ns
2022	Aboveground	<0.001	<0.01	<0.001
	Roots	<0.001	<0.01	<0.001

Table 11S. Average N release rate (kg ha^{-1}) (emmeans) from CCs aboveground residues decomposition in 2020. Standard Error (SE); upper and lower confidence interval (CI) (at 0.95 confidence interval). Different letters indicate significant differences ($p<0.05$) between the levels of the source of variation.

Time	**Treatment**	**emmean**	**SE**	**lower.CI**	**upper.CI**	**Significance (p<0.05)**
April	Succession	0.05036667	0.00386797	0.03806829	0.06266504	b
April	Fixed	0.01536667	0.00386797	0.00306829	0.02766504	cd
April	NoCCs	0.00573333	0.00386797	-0.00656504	0.01803171	d
May	Succession	0.07726667	0.00386797	0.06496829	0.08956504	a
May	Fixed	0.01476667	0.00386797	0.00246829	0.02706504	cd
May	NoCCs	0.01156667	0.00386797	-0.00073171	0.02386504	cd
June	Succession	0.096	0.00386797	0.08370162	0.10829838	a
June	Fixed	0.02186667	0.00386797	0.00956829	0.03416504	cd
June	NoCCs	0.01243333	0.00386797	0.00013496	0.02473171	cd
July	Succession	0.03226667	0.00386797	0.01996829	0.04456504	bc

July	Fixed	0.01003333	0.00386797	-0.00226504	0.02233171	d
July	NoCCs	0.0041	0.00386797	-0.00819838	0.01639838	d
August	Succession	0.02013333	0.00386797	0.00783496	0.03243171	cd
August	Fixed	0.00796667	0.00386797	-0.00433171	0.02026504	d
August	NoCCs	0.00183333	0.00386797	-0.01046504	0.01413171	d

Table 12S. Average N release rate (kg ha^{-1}) (emmeans) from CCs aboveground residues decomposition in 2021. Standard Error (SE); upper and lower confidence interval (CI) (at 0.95 confidence interval). Different letters indicate significant differences ($p<0.05$) between the levels of the source of variation.

Groups	**Treatment**	**emmean**	**SE**	**lower.CI**	**upper.CI**	**Significance (p<0.05)**
April	NoCCs	0.29006667	0.01625309	0.23838927	0.34174407	a
April	Succession	0.28023333	0.01625309	0.22855593	0.33191073	a
April	Fixed	0.10893333	0.01625309	0.05725593	0.16061073	cde
May	NoCCs	0.27193333	0.01625309	0.22025593	0.32361073	a
May	Succession	0.2112	0.01625309	0.1595226	0.2628774	ab
May	Fixed	0.1352	0.01625309	0.0835226	0.1868774	bcd
June	NoCCs	0.15153333	0.01625309	0.09985593	0.20321073	bc
June	Succession	0.11123333	0.01625309	0.05955593	0.16291073	cde
June	Fixed	0.0821	0.01625309	0.0304226	0.1337774	cdef
July	NoCCs	0.0484	0.01625309	-0.0032774	0.1000774	def
July	Succession	0.0363	0.01625309	-0.0153774	0.0879774	ef
July	Fixed	0.0274	0.01625309	-0.0242774	0.0790774	ef
August	NoCCs	0.02403333	0.01625309	-0.02764407	0.07571073	ef
August	Succession	0.01883333	0.01625309	-0.03284407	0.07051073	f
August	Fixed	0.0143	0.01625309	-0.0373774	0.0659774	f

Table 13S. Average N release rate (kg ha^{-1}) (emmeans) from CCs aboveground residues decomposition in 2022. Standard Error (SE); upper and lower confidence interval (at 0.95 confidence interval). Different letters indicate significant differences ($p<0.05$) between the levels of the source of variation.

Groups	**Treatment**	**emmean**	**SE**	**lower.CI**	**upper.CI**	**Significance (p<0.05)**
April	NoCCs	0.24286667	0.01788938	0.18544341	0.30028993	ab
April	Succession	0.17613333	0.01788938	0.11871007	0.23355659	bc
April	Fixed	0.0253	0.01788938	-0.03212326	0.08272326	d
May	NoCCs	0.2992	0.01788938	0.24177674	0.35662326	a

May	Succession	0.16383333	0.01788938	0.10641007	0.22125659	bc
May	Fixed	0.04133333	0.01788938	-0.01608993	0.09875659	d
June	NoCCs	0.16816667	0.01788938	0.11074341	0.22558993	bc
June	Succession	0.07786667	0.01788938	0.02044341	0.13528993	cd
June	Fixed	0.02783333	0.01788938	-0.02958993	0.08525659	d
July	NoCCs	0.06253333	0.01788938	0.00511007	0.11995659	d
July	Succession	0.02786667	0.01788938	-0.02955659	0.08528993	d
July	Fixed	0.01203333	0.01788938	-0.04538993	0.06945659	d
August	Succession	0.07515	0.02190993	0.00482116	0.14547884	cd
August	NoCCs	0.031	0.02190993	-0.03932884	0.10132884	d
August	Fixed	0.007	0.02190993	-0.06332884	0.07732884	d

Table 14S. Average N release rate (kg ha^{-1}) (emmeans) from CCs roots residues decomposition in 2020. Standard Error (SE); upper and lower confidence interval (CI) (at 0.95 confidence interval). Different letters indicate significant differences (p<0.05) between the levels of the source of variation.

Groups	Treatment	emmean	SE	lower.CI	upper.CI	Significance (p<0.05)
April	NoCCs	0.0085	0.00290589	-0.00082764	0.01782764	bc
April	Succession	-0.00116667	0.00290589	-0.0104943	0.00816097	cd
April	Fixed	-0.02776667	0.00290589	-0.0370943	-0.01843903	e
May	Succession	0.01563333	0.00290589	0.0063057	0.02496097	b
May	NoCCs	0.01243333	0.00290589	0.0031057	0.02176097	bc
May	Fixed	-0.01553333	0.00290589	-0.02486097	-0.0062057	de
June	Succession	0.03436667	0.00290589	0.02503903	0.0436943	a
June	NoCCs	0.01326667	0.00290589	0.00393903	0.0225943	bc
June	Fixed	0.008	0.00290589	-0.00132764	0.01732764	bc
July	Succession	0.02016667	0.00290589	0.01083903	0.0294943	ab
July	Fixed	0.01376667	0.00290589	0.00443903	0.0230943	bc
July	NoCCs	0.0043	0.00290589	-0.00502764	0.01362764	bc
August	Succession	0.006	0.00355897	-0.00542398	0.01742398	bc
August	NoCCs	0.0028	0.00355897	-0.00862398	0.01422398	bc
August	Fixed	-0.0218	0.00355897	-0.03322398	-0.01037602	e

Table 15S. Average N release rate (kg ha^{-1}) (emmeans) from CCs roots residues decomposition in 2021. Standard Error (SE); upper and lower confidence interval (CI) (at 0.95 confidence interval). Different letters indicate significant differences (p<0.05) between the levels of the source of variation.

Groups	Treatment	emmean	SE	df	lower.CI	upper.CI	Significance (p<0.05)
April	NoCCs	0.07926667	0.00829387	27	0.05264411	0.10588923	cd
April	Succession	0.06126667	0.00829387	27	0.03464411	0.08788923	de
April	Fixed	-0.07153333	0.00829387	27	-0.09815589	-0.04491077	fg
May	NoCCs	0.15666667	0.00829387	27	0.13004411	0.18328923	ab
May	Succession	0.07853333	0.00829387	27	0.05191077	0.10515589	cd

May	Fixed	-0.0934	0.00829387	27	-0.12002256	-0.06677744	g
June	NoCCs	0.17166667	0.00829387	27	0.14504411	0.19828923	a
June	Succession	0.05646667	0.00829387	27	0.02984411	0.08308923	de
June	Fixed	-0.07656667	0.00829387	27	-0.10318923	-0.04994411	fg
July	NoCCs	0.11506667	0.00829387	27	0.08844411	0.14168923	bc
July	Succession	0.02536667	0.00829387	27	-0.00125589	0.05198923	e
July	Fixed	-0.04126667	0.00829387	27	-0.06788923	-0.01464411	f
August	NoCCs	0.0779	0.01015788	27	0.04529416	0.11050584	cd
August	Succession	0.0277	0.01015788	27	-0.00490584	0.06030584	de
August	Fixed	-0.0351	0.01015788	27	-0.06770584	-0.00249416	f

Table 16S. Average N release rate (kg ha^{-1}) (emmeans) from CCs roots residues decomposition in 2022. Standard Error (SE); upper and lower confidence interval (CI) (at 0.95 confidence interval). Different letters indicate significant differences ($p<0.05$) between the levels of the source of variation.

Groups	Treatment	emmean	SE	df	lower.CI	upper.CI	Significance (p<0.05)
April	NoCCs	0.07926667	0.00829387	27	0.05264411	0.10588923	cd
April	Succession	0.06126667	0.00829387	27	0.03464411	0.08788923	de
April	Fixed	-0.07153333	0.00829387	27	-0.09815589	-0.04491077	fg
May	NoCCs	0.15666667	0.00829387	27	0.13004411	0.18328923	ab
May	Succession	0.07853333	0.00829387	27	0.05191077	0.10515589	cd
May	Fixed	-0.0934	0.00829387	27	-0.12002256	-0.06677744	g
June	NoCCs	0.17166667	0.00829387	27	0.14504411	0.19828923	a
June	Succession	0.05646667	0.00829387	27	0.02984411	0.08308923	de
June	Fixed	-0.07656667	0.00829387	27	-0.10318923	-0.04994411	fg
July	NoCCs	0.11506667	0.00829387	27	0.08844411	0.14168923	bc
July	Succession	0.02536667	0.00829387	27	-0.00125589	0.05198923	e
July	Fixed	-0.04126667	0.00829387	27	-0.06788923	-0.01464411	f
August	NoCCs	0.0779	0.01015788	27	0.04529416	0.11050584	cd
August	Succession	0.0277	0.01015788	27	-0.00490584	0.06030584	de
August	Fixed	-0.0351	0.01015788	27	-0.06770584	-0.00249416	f

Table 17S. Soil water content source of variation in 0-20 and 20-40cm soil depths.* Significance; ns = not significant (Wald test ANOVA).

Year	Depth (cm)	Source of variation	p value*
2020	0-50	Treatment	ns
		Time	<0.001
		Time x Treatment	ns
	50-100	Treatment	ns
		Time	<0.001
		Treatment x Time	ns

2021	0-50	Treatment	<0.05
		Time	<0.001
		Treatment x Time	ns
	50-100	Treatment	<0.01
		Time	<0.001
		Treatment x Time	ns
2022	0-50	Treatment	<0.01
		Time	<0.001
		Treatment x Time	ns
	50-100	Treatment	<0.01
		Time	<0.001
		Treatment X Time	ns

Table 18S. Soil water content average values (emmeans) at 0-50 cm depth during cash crop seasons of 2020. Standard Error (SE); upper and lower confidence interval (CI) (at 0.95 confidence interval). Different letters indicate significant differences ($p<0.05$) between the levels of the source of variation.

Data	**emmean**	**SE**	**df**	**lower.CI**	**upper.CI**	**Significance (p<0.05)**
2020-06-01	-3.078033	5.20712497	108	-18.2812653	12.1251993	c
2020-06-05	15.2300047	5.20712497	108	0.02677234	30.433237	abc
2020-06-12	35.5993308	5.20712497	108	20.3960984	50.8025631	a
2020-06-22	27.0658155	5.20712497	108	11.8625832	42.2690478	ab
2020-06-29	11.6307204	5.20712497	108	-3.57251191	26.8339527	abc
2020-07-03	6.93440492	5.20712497	108	-8.26882741	22.1376372	bc
2020-07-08	11.7650029	5.20712497	108	-3.43822941	26.9682352	abc
2020-07-14	-0.27504625	5.20712497	108	-15.4782786	14.9281861	c
2020-08-05	14.8354264	5.20712497	108	-0.36780591	30.0386587	abc
2020-08-26	11.8775942	5.20712497	108	-3.32563816	27.0808265	abc
2020-09-30	-7.89276733	5.20712497	108	-23.0959997	7.31046499	c
2020-10-07	-0.36303067	5.20712497	108	-15.566263	14.8402017	c

Table 19S. Soil water content average values (emmeans) at 50-100 cm depth during cash crop seasons of 2020. Standard Error (SE); upper and lower confidence interval (CI) (at 0.95 confidence interval). Different letters indicate significant differences ($p<0.05$) between the levels of the source of variation.

Data	**emmean**	**SE**	**df**	**lower.CI**	**upper.CI**	**Significance (p<0.05)**
2020-06-12	18.4250751	4.53469077	108	5.18514752	31.6650026	a
2020-06-22	18.3822567	4.53469077	108	5.14232918	31.6221843	a
2020-06-29	9.64373264	4.53469077	108	-3.59619491	22.8836602	ab
2020-07-03	4.02400819	4.53469077	108	-9.21591936	17.2639357	abc
2020-07-08	2.22986868	4.53469077	108	-11.0100589	15.4697962	abc
2020-06-05	-1.55821785	4.53469077	108	-14.7981454	11.6817097	abc

2020-06-01	-1.70156396	4.53469077	108	-14.9414915	11.5383636	abc
2020-07-14	-8.0274566	4.53469077	108	-21.2673842	5.21247096	bc
2020-08-05	-10.4578744	4.53469077	108	-23.6978019	2.78205318	bc
2020-08-26	-15.1348137	4.53469077	108	-28.3747412	-1.89488613	c
2020-10-07	-15.6603817	4.53469077	108	-28.9003093	-2.42045418	c
2020-09-30	-17.49817	4.53469077	108	-30.7380976	-4.25824245	c

Table 20S. Soil water content average values (emmeans) at 0-50 cm depth during cash crop seasons of 2021. Standard Error (SE); upper and lower confidence interval (CI) (at 0.95 confidence interval). Different letters indicate significant differences ($p<0.05$) between the levels of the source of variation.

Data	**emmean**	**SE**	**lower.CI**	**upper.CI**	**Significance (p<0.05)**
2021-04-14	32.2346959	3.98337076	19.9447132	44.5246786	a
2021-04-20	17.2596869	3.98337076	4.96970424	29.5496696	abc
2021-04-29	10.1646694	3.98337076	-2.12531326	22.4546521	bcd
2021-05-05	14.8093402	3.98337076	2.51935749	27.0993228	abc
2021-05-14	33.8645111	3.98337076	21.5745284	46.1544938	a
2021-05-20	25.2466478	3.98337076	12.9566652	37.5366305	ab
2021-05-26	32.9000352	3.98337076	20.6100526	45.1900179	a
2021-06-10	6.34695325	3.98337076	-5.94302942	18.6369359	bcde
2021-06-15	-1.33983342	3.98337076	-13.6298161	10.9501493	cdefg
2021-06-23	-8.5165995	3.98337076	-20.8065822	3.77338317	defg
2021-06-30	-8.96022917	3.98337076	-21.2502118	3.32975351	defg
2021-07-09	2.57746908	3.98337076	-9.71251359	14.8674518	cdef
2021-07-15	-12.6922853	3.98337076	-24.9822679	-0.40230258	efgh
2021-07-22	-17.0701588	3.98337076	-29.3601415	-4.78017616	fghi
2021-07-28	-20.5275591	3.98337076	-32.8175418	-8.23757641	ghij
2021-08-25	-40.8781834	3.98337076	-53.1681661	-28.5882007	jk
2021-09-03	-44.4067829	3.98337076	-56.6967656	-32.1168002	k
2021-09-10	-47.1168511	3.98337076	-59.4068338	-34.8268684	k
2021-09-18	-43.5369403	3.98337076	-55.826923	-31.2469577	k
2021-09-23	-39.8520746	4.19884145	-52.8068538	-26.8972954	jk
2021-10-02	-31.8866618	4.19884145	-44.841441	-18.9318826	hijk
2021-10-07	-36.4446845	4.19884145	-49.3994637	-23.4899053	ijk

Table 21S. Soil water content average values (emmeans) at 50-100 cm depth during cash crop seasons of 2021. Standard Error (SE); upper and lower confidence interval (CI) (at 0.95 confidence interval). Different letters indicate significant differences ($p<0.05$) between the levels of the source of variation.

Data	**emmean**	**SE**	**lower.CI**	**upper.CI**	**Significance (p<0.05)**
2021-05-26	40.127579	2.89261019	31.2029443	49.0522138	a
2021-05-20	17.9378542	2.89261019	9.01321949	26.862489	b
2021-05-14	16.6843077	2.89261019	7.75967297	25.6089425	b
2021-06-10	10.4386453	2.89261019	1.51401054	19.36328	bc
2021-04-14	8.08247542	2.89261019	-0.84215933	17.0071102	bcd
2021-05-05	7.54300861	2.89261019	-1.38162613	16.4676434	bcd
2021-04-20	7.12079139	2.89261019	-1.80384335	16.0454261	bcd
2021-04-29	4.84227167	2.89261019	-4.08236308	13.7669064	bcd
2021-06-15	4.7835991	2.89261019	-4.14103565	13.7082338	bcd
2021-06-23	1.12489813	2.89261019	-7.79973662	10.0495329	cde
2021-06-30	-1.88408493	2.89261019	-10.8087197	7.04054981	cdef
2021-07-09	-5.19033583	2.89261019	-14.1149706	3.73429891	defg
2021-07-15	-14.2605547	2.89261019	-23.1851894	-5.33591991	efg
2021-07-22	-15.7164297	2.89261019	-24.6410645	-6.79179498	fg
2021-07-28	-17.7999296	2.89261019	-26.7245643	-8.87529484	g
2021-09-03	-38.8514852	2.89261019	-47.77612	-29.9268505	h
2021-08-25	-39.4784269	2.89261019	-48.4030616	-30.5537921	h
2021-09-23	-39.7708942	3.04907886	-49.1782852	-30.3635032	h
2021-09-10	-40.0235733	2.89261019	-48.948208	-31.0989385	h
2021-09-18	-41.1018334	2.89261019	-50.0264681	-32.1771987	h
2021-10-02	-41.4364187	3.04907886	-50.8438097	-32.0290277	h
2021-10-07	-41.9030443	3.04907886	-51.3104354	-32.4956533	h

Table 22S. Soil water content average values (emmeans) at 0-50 cm depth during cash crop seasons of 2022. Standard Error (SE); upper and lower confidence interval (CI) (at 0.95 confidence interval). Different letters indicate significant differences ($p<0.05$) between the levels of the source of variation.

Data	**emmean**	**SE**	**lower.CI**	**upper.CI**	**Significance (p<0.05)**
2022-04-14	-12.4211568	4.75791673	-27.0378665	2.19555287	abcd
2022-04-20	-15.7402027	4.75791673	-30.3569124	-1.12349297	abcde
2022-04-28	-3.83993617	4.75791673	-18.4566459	10.7767735	a
2022-05-04	-12.1553265	4.75791673	-26.7720362	2.4613832	abcd
2022-05-12	-8.45451858	4.75791673	-23.0712283	6.16219112	abc
2022-05-19	-13.0954753	4.75791673	-27.7121849	1.52123445	abcd
2022-05-25	-11.9928848	4.75791673	-26.6095945	2.62382487	abcd
2022-05-31	-14.6123795	4.75791673	-29.2290892	0.0043302	abcd
2022-06-09	-6.77314275	4.75791673	-21.3898524	7.84356695	ab
2022-06-15	-9.508019	4.75791673	-24.1247287	5.1086907	abc
2022-06-22	-14.4090003	4.75791673	-29.0257099	0.20770945	abcd
2022-06-28	-16.3506853	4.75791673	-30.9673949	-1.73397555	abcde
2022-07-05	-19.7290799	4.75791673	-34.3457896	-5.11237022	abcde
2022-07-14	-13.7229072	4.75791673	-28.3396169	0.89380253	abcd
2022-07-21	-17.6646641	4.75791673	-32.2813738	-3.04795438	abcde
2022-07-28	-30.1209038	4.75791673	-44.7376135	-15.5041941	bcde
2022-08-04	-35.4232278	4.75791673	-50.0399375	-20.8065181	de
2022-08-09	-32.9636268	4.75791673	-47.5803364	-18.3469171	cde
2022-08-24	-20.1919272	4.75791673	-34.8086369	-5.57521747	abcde
2022-08-31	-22.3631827	4.75791673	-36.9798924	-7.74647297	abcde
2022-09-07	-40.1124445	4.75791673	-54.7291542	-25.4957348	e

Table 23S. Soil water content average values (emmeans) at 50-100 cm depth during cash crop seasons of 2022. Standard Error (SE); upper and lower confidence interval (CI) (at 0.95 confidence interval). Different letters indicate significant differences (p<0.05) between the levels of the source of variation.

Data	**emmean**	**SE**	**lower.CI**	**upper.CI**	**Significance (p<0.05)**
2022-04-14	-3.14353451	4.47010871	-16.8760744	10.5890054	a
2022-05-25	-5.5935025	4.47010871	-19.3260424	8.1390374	a
2022-06-15	-5.74835042	4.47010871	-19.4808903	7.98418948	a
2022-06-22	-6.10192611	4.47010871	-19.834466	7.63061379	a
2022-06-09	-6.53153007	4.47010871	-20.26407	7.20100983	a
2022-05-31	-6.63330903	4.47010871	-20.3658489	7.09923087	a
2022-05-12	-6.6520891	4.47010871	-20.384629	7.0804508	a
2022-04-28	-6.68552042	4.47010871	-20.4180603	7.04701948	a
2022-04-20	-6.75706493	4.47010871	-20.4896048	6.97547497	a
2022-05-19	-6.83008062	4.47010871	-20.5626205	6.90245927	a
2022-06-28	-6.90907972	4.47010871	-20.6416196	6.82346017	a
2022-05-04	-7.48820167	4.47010871	-21.2207416	6.24433823	a
2022-07-05	-7.54675299	4.47010871	-21.2792929	6.18578691	a
2022-07-14	-9.00672375	4.47010871	-22.7392636	4.72581615	a
2022-07-21	-10.7056263	4.47010871	-24.4381661	3.02691365	a
2022-07-28	-15.2305136	4.47010871	-28.9630535	-1.49797371	ab
2022-08-04	-17.7316668	4.47010871	-31.4642067	-3.99912691	ab
2022-08-09	-21.441254	4.47010871	-35.1737939	-7.70871406	ab
2022-08-31	-22.0814856	4.47010871	-35.8140255	-8.34894566	ab
2022-08-24	-23.2617977	4.47010871	-36.9943376	-9.52925781	ab
2022-09-07	-36.8449938	4.47010871	-50.5775337	-23.1124539	b

Chapter 3

Seasonal dynamics of water availability and N cycle under cover crops

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Abstract

Cover crops (CCs) exhibit pronounced interplay with the soil water content (SWC) and nitrogen (N) cycle. However, their impact on both resources may vary throughout crop succession seasons. This seasonal variability is often not captured by research studies where a single time point sample is taken. The study aims to investigate how the initial introduction of diverse CCs affects the seasonal variability of both soil chemical (NO_3^-) and biological (soil N functional genes - NFGs) constituents of the N cycle, the SWC, and the cash crop yield in a three-year maize-soybean succession in northeastern Italy. Three CCs managements were compared: a fixed treatment with triticale; a 3-year succession of rye, crimson clover, and mustard; and a control with no CCs. No differences in the cash crop yields were observed among CCs treatments. All CCs didn't exhibit water competition with ensuing cash crops compared to control. At CCs termination time, grasses reduced soil NO_3^- (catch crops) and stimulated potential microbial N-fixation activity (nifH), whereas clover measured the highest residual NO_3^- and potential N nitrification (AOA). Agronomic operations disrupted differences in NFGs abundances after CC residue incorporation. During the cash crop season higher N release was estimated by the CC-NCALC model for clover, mustard, and weeds (with wild leguminous) compared to rye and triticale (immobilization). Nevertheless, consistent N nitrification and denitrification potential were observed in all treatments (except for the weed-free bare soil), with greater NFGs abundance when cultivating soybean instead of maize. This underscored the role of cash crop species in shaping N transformation dynamics. The observed pronounced seasonal variability highlights how the effective utilization of NFGs and chemical indicators to evaluate the impact of CCs on N dynamics and SWC requires careful consideration of the timing of sample collection within a crop succession (e.g. termination time is recommended for CCs).

Keywords: cover crop management; soil health; soil N functional genes; soil water content

1. Introduction

The introduction of cover crops (CCs) has been linked to significant changes in key elements of agroecosystems like nitrogen (N) cycling encompassing both its chemical and biological determinants (Kaspar and Singer, 2011) and soil water content (SWC) (Malone et al., 2007). The introduction of CCs holds the potential of mitigating N losses (Tully and Ryals, 2017) while concurrently enhancing fertilization (Dabney et al., 2001; Gabriel et al., 2016) and irrigation (Nowak et al., 2022) management efficiency. However, these potential advantages exhibit heterogeneity contingent upon the temporal progression of distinct phases such as the growth period of CCs, their termination, and the subsequent integration of their residues into the upper soil layer (Hu et al., 2023a). This seasonal variability, which might affect the SWC dynamics and the driving forces of N transformations, is often not captured by research studies where a single time point sample is taken (Kaspar and Singer, 2011; Hu et al., 2023a).

In response to this constraint, a systematic inquiry into the effects of CCs on both SWC and N dynamics at critical phases within their life cycle emerges as a matter of heightened significance, aimed at the optimization of the advantages derived from the integration of CCs into agricultural cropping systems. Specifically, a comprehensive assessment of the temporal evolution of SWC and N dynamics can provide a number of different useful pieces of information, which vary according to the choice of some key phases of the CC-cash crop succession, as follows: (i) the analysis from the initiation of CC sowing to their termination shows the potential impact of different species during their growing phase; (ii) the period between CC termination and 1.5 months after offers a promising avenue for scrutinizing the multifaceted implications of leaving the residues on the field (incorporated or on the surface) as evidenced by previous research (Poffenbarger et al., 2015; Raimondi et al., 2023a); (iii) the cash crop growing period yields valuable insights into the interplay between CC residues and the growth of the cash crop, along with its potential repercussions on the cash crops' yield quantity and quality.

The introduction of CCs within fallow intervals of a crop rotation has been demonstrated to enhance various soil physical properties (Blanco-Canqui et al., 2020) which translates into an augmented availability of SWC for the ensuing cash crop (Malone et al., 2007). However, it is worth noting that the growth of CCs, in contrast, leads to the utilization of soil water, potentially diminishing water accessibility for the subsequent cash crop (Krueger et al., 2011; Meyer et al., 2020). The influence of these factors is contingent upon the specific CC type, its growth rate, and the prevalent environmental conditions, thereby giving rise to variable degrees of impact. In certain instances, substantial water competition emerges, risking compromising the final yield production and quality as reported in early investigations of Alonso-Ayuso et al. (2014), Alvarez et al. (2017), and Gabriel et al. (2016, 2019).

The impact of CCs on N-cycling, which is intricately governed by the network of activities performed by N-cycling microbial communities, has been examined in previous studies through the analysis of the biological determinants, complemented by the quantitative assessment of soil mineral N (NO_3^- and NH_4^+) content (Kong et al., 2010; Norton et al., 2015; Ouyang et al., 2016). N-cycling microbial populations and functions can be perturbed by alterations in the soil environment resulting from management practices such as CCs introduction (Behnke et al., 2022; Kim et al., 2022a). Consequently, the evaluation of genes encoding crucial enzymes responsible for soil N transformations has been employed to monitor N-related processes like fixation, nitrification, and denitrification after CCs introduction (Hu et al., 2021). The commonly used N-cycling functional genes (NFGs) include *nifH* (nitrogenase; N-fixation), ammonia-oxidizing archaea (AOA) and bacterial (AOB) *amoA* (ammonia monooxygenase; nitrification), *nirK* (nitrite reductase; intermediate-stage denitrification), and *nosZ* (nitrous oxide reductase; terminal-stage denitrification) (Hirsch and Mauchline, 2015). These genes' abundance and expression reflect the sizes and activities of microbial communities engaged in specific N cycling activities (Wang et al., 2018), and they tend to correlate closely with inorganic N products (Lourenço et al., 2022).

All these genes, and the relative N processes, as well as the SWC and soil mineral N content, exhibit a robust interconnection among themselves and they can all be differently affected by the CCs introduction in each rotation period concurrently with the inherent attributes of the specific CCs species, agricultural management, soil type, elevation, and local and annual climate variability (Hively et al., 2020; Lee et al., 2016; Poeplau and Don, 2015). Several studies assessed the effect of different CCs species during their growing season, on the soil preferential N transformation processes and the soil NFGs abundance, in conjunction with SWC (Li et al., 2017; Momesso et al., 2022), the residual soil mineral N (Linton et al., 2020) and the interaction with both of them (Nadeau et al., 2019; You et al., 2022). While several studies have documented the influence of CC residues on soil macro and micro fauna (Londoño-R et al., 2013), limited investigations have been devoted to study the effect of incorporating CCs residues on specific soil NFGs including AOA *amoA*, AOB *amoA*, *nosZ*, *nirK*, and *nifH*.

Similarly, the extent to which CCs residues can contribute to the cash crop's N requirements and thereby mitigate the dependence on N fertilizers (Wittwer et al., 2020), or irrigation input (Steele et al., 2012) during the cash crop growing season, remain uncertain. Moreover, the influence of fertilization levels and strategies, irrigation interventions (Wolsing and Prieme, 2004), tillage practices, and the selection of both CCs and subsequent cash crops (Behnke et al., 2020), has been documented to impact the dynamics of microbial guilds encompassing nitrifiers, denitrifiers, and N fixers. Precipitation patterns also hold a crucial role in shaping the abundance of soil NFGs and associated N processes. Particularly, it has been observed that the sensitivity of soil N cycling to decreased rainfall is more pronounced in humid regions than in arid regions (Wu et al., 2022).

To sum up, it can be stated that the use of CCs is an agricultural practice with a pronounced interplay with water and N cycles within cropping systems. They can compete for these resources with cash crops, thereby risking impairing their final yield, or conversely being a source of N nutrient while increasing the SWC. Given that CCs adoption is typically pursued by farmers with economic success as a foremost priority (Bergtold et al., 2019), a comprehensive investigation into the seasonal effects of CCs on these resources becomes imperative. This aims to optimize the benefits of CCs while mitigating any detrimental consequences they might entail.

Within this framework, the objective of the present study is to investigate the impact of the initial introduction of diverse CCs within a maize-maize-soybean sequence, on the chemical and biological constituents of the N cycle, alongside the SWC, and the cash crop yield. The study's scope further involves discerning the interrelationships among these variables across distinct phases of the crop succession, to comprehensively capture the seasonal variability.

2. Materials and methods

2.1 Site description

The research was conducted from October 2019 to October 2022 in a site of about 5.5 ha located in the experimental farm "L. Toniolo" in Legnaro (PD), north-eastern Italy (45°20′53″ N, 11°57′11″ E, 6 m a.s.l.). The area is situated in a plain region of fluvial origin with a soil classified as Fulvi-calcaric Cambisol (WRBSR, 2014). The climate is humid subtropical (Cfa class in Köppen classification) (Rubel et al., 2017) with excess water in autumn and spring and water stress in summer. The annual average (1992-2022) rainfall and temperature are 830 mm and 13.9 °C, respectively. The annual average minimum temperature is 8.7 °C and the month with the lowest temperature is January (−0.15 °C on average), whereas the maximum annual average temperature is 18.6 °C and the month with the maximum temperature is July (29.5 °C on average). All the weather data for the present study were downloaded from the meteorological station of the Veneto regional agency for environmental protection (ARPAV), located within the experimental site. The upper soil layer analyzed in the present study (0-40 cm depth) has a loamy-silty loam texture with 37% of sand, 44% of silt, and 19% of clay. The soil organic matter content was 1.4 %, the total Kjeldahl N of 0.9 g kg^{-1}, and inorganic N (NO_3^--N) of 57 mg kg^{-1}. The soil hydrological properties for the first 2-m depth soil layers are reported in detail by Tolomio and Borin (2019) and showed on average 45.8% of saturated water content (SAT), 33.9% of drained upper limit (DUL) and 13.4% of permanent wilting point (PWP).

2.2 Experimental layout and crop management

The experimental framework employed a randomized split-plot design comprising 2 Blocks and 3 CC management strategies allocated to the main plots. The experiment was conducted in a 3-year winter CCs-cash crop sequence (2019/2020 – I year; 2020/2021 – II year; 2021/2022 – III year). The 3 winter CCs managements included: a fixed treatment (Fixed) with triticale (X *triticosecale*) cultivated for all three years; a succession treatment (Succession) with 3 CCs species (*Secale cereale* L. – in the I year; *Trifolium incarnatum* L. – in the II year; *Sinapis alba* L. – in the III year); and a control (NoCCs) with no CCs and no weeds control. The CCs were sown on October 10th 2019, October 9th 2020, and September 24th 2021, and terminated each year on March 31th by shredding with a rotary mulcher followed by residues incorporation in the upper soil layer. The cash crops were silage maize (*Zea mays* L.) in the I and II years and soybean (*Glycine max* (L.) Merr.) in the III year. They were sown on April 17th 2020, April 26th 2021, and May 10th 2022 and harvested on August 25th 2020 and 2021, and on October 13th 2022.

The tillage operations consisted of subsoil tillage (30 cm depth) after the CCs termination and rolling harrow for the cash crop seedbed preparation. Maize was fertilized with 200 kg N ha^{-1} (16% of urea before sowing and the rest as top-dressing on May 25th 2020 and May 29th 2021), 80 kg P_2O_5 ha^{-1}, and 80 kg K_2O ha^{-1} before sowing; soybean received only 46 kg P_2O_5 ha^{-1} before sowing. Irrigation was applied once each year during the cash crop cycle, with 40 mm in 2020, 30 mm in 2021 and 40 mm in 2022. Weeds were chemically controlled before and mechanically controlled after cash crops emergence, in all the 3 years.

2.3 Samples collection

Crop and soil parameters were investigated, within each experimental year, in a way to enable the delineation of 3 distinct temporal periods: (i) the first extending from the CC sowing to termination (WI - Winter Period) to evaluate the evolution of the parameters under analysis during the CCs growing season; (ii) the second extending from the CC termination to 1.5 months after (SP - Spring Period). The period was determined based on literature findings (Kuo and Sainju, 1998; Lawson et al., 2013; Poffenbarger et al., 2015) which presented the mean timeframe for the peak occurrence of N release rates from CCs biomass after termination; (iii) the third extending from the conclusion of the previous period until the cash crop harvest (SU- Summer Period).

2.3.1 Soil variables

Soil samples were collected in 3 georeferenced sampling points within each plot, using a drill to extract them at two depths (0-20 and 20-40 cm). The sampling campaigns were executed during each experimental year at CCs sowing and termination (for the WI period), 1.5 months after the CCs termination (for the SP period), and at cash crop harvest (for the SU period). The soil samples collected in the field were left to air-dry for about 1 month before being sifted (2-mm sieve) and then stored in falcon tubes before the analysis of the NO_3^--N content (Nmin) and the soil NFGs abundance. The soil Nmin content was only measured at CCs sowing and termination, and at cash crop harvest time. All the NFGs and Nmin values are reported from here on out as the delta (Δ) between the values measured at the beginning and the end of each period under analysis.

SWC was measured on a weekly basis (± 4 days) in a sample point within each plot using Sentek's Diviner2000 capacitance sensor (Sentek Environmental Technologies, Kent Town, South Australia). As for the soil chemical and biological characteristics, SWC is reported for the 0-20 and 20-40 cm depths. The measurements were collected throughout the whole experimental period starting from May 25th, 2020. All the SWC values are reported from now on as the Δ between each value measured in each monitoring date and the initial SWC values measured at the beginning of the measurements (May 25th 2020).

2.3.2 Cash crops parameters

The cash crops' aboveground biomass was collected each year at harvest time from three sampling areas in each plot of 18 m^2 for maize (whole plant at the silage stage) and 13.5 m^2 for soybean (grain). All the biomass samples were weighed for their fresh weight and dry matter content after being dried in a thermos-ventilated oven (65°C).

2.4 Soil functional genes analyses

2.4.1 DNA extraction and quantification

The protocol was based on a report from Chiodi et al. (2019). Briefly, soil samples were ground with a mortar and sifted with a mesh of 500 microns. 0.3 g of soil was used for total DNA extraction which was performed adding 600 µL of NaP as extracting buffer with two types of glass beads (having different diameters) used to facilitate the cells' lysis. A TissueLyser II instrument (Qiagen, Germany) was used to homogenize the samples during the lysis process (30 Hz for 4 minutes). After the crude DNA extraction, the samples were centrifugated for 5 minutes before being purified with the automatized system BioSprint96 (Qiagen, Germany). Each box of the extraction plate was prepared with 200 µL of isopropanol, 500 µL of RPW extraction buffer, 500 µL of ethanol 96%, and the addition of 500 µL of the non-ionic surfactant TWEEN 20 (Merck, Germany). 100 µL of sterilized water was added for the DNA elution, 200 µL of a supernatant sample, and 25 µL of MagAttract suspension G (Qiagen, Germany) magnetic beads.

The extracted DNA was quantified with the Qubit 3.0 fluorometer (Thermo Fisher Scientific, USA) using the Qubit 1x dsDNA High Sensitivity Assay Kit (Thermo Fisher Scientific, USA) according to the manufacturer's guidelines. The purified DNA was stored at -20 °C.

2.4.2 PCR amplification primers

The primers used in this study are archaeal *amoA* F (STAATGGTCTGGCTTAGACG) and archaeal *amoA* R (GCG GCC ATC CAT CTG TAT CT) (Francis et al., 2005), bacterial *amoA* F (GGGGTTTCTACTGGTGGT) and bacterial *amoA* R (CCCCTCKGSAAAGCCTTCTTC) (Rotthauwe *et al.*, 1997), *nosZ* F (CGYTGTTCMTCGACAGCCAG) and *nosZ* R (CATGTGCAGNGCRTGGCAGAA) (Rösch et al., 2002), *nifH* F (AAAGGYGGWATCGGYAARTCCACCAC) and *nifH* R (TTGTTSGCSGCRTACATSGCCATCAT) (Rösch et al., 2002), *nirK* F (ATYGGCGGVCAYGGCGA) and *nirK* R (RGCCTCGATCAGRTTRTGGTT) (Henry et al., 2004). The *nosZ* gene primer couple used in this study is targeting the clade I.

2.4.3 Quantitative Real-Time PCR

QuantitativeReal-Time PCR (qPCR) analyses were performed using the QuantStudio 5 platform (Thermo Fisher Scientific, USA). Each sample was analysed using 3 biological replicates and performing on each 3 technical replicates. The reaction mix was composed by 1 µL of template DNA (diluted with the ratio1:20 using PCR-grade water), 2.5 µL of PowerUp SYBR Green Master Mix (Thermo Fisher Scientific, USA), 0.15 µL of forward primer, 0.15 µL of reverse primer, and 1.2 µL of PCR-grade water. The thermal cycling conditions were: hold stage 50 °C for 2 minutes and 95 °C for 15 seconds (1 cycle), PCR stage 95 °C for 15 seconds and 60 °C for 1 minute (40 cycles), Melt Curve stage 95 °C for 15 seconds, 60 °C for 1 minute and 95 °C for 15 seconds.

The results obtained with the qPCR analysis are expressed as the number of cycles required to reach the threshold (Ct) at which the instrument starts to detect a fluorescent signal. Ct values are inversely proportional to the amount of target present in the sample, e.g. a high Ct value corresponds to a small amount of target gene in the sample. The Ct values have been transformed into gene copy numbers using previously obtained calibration curves (Sims et al., 2012; Zanardo et al., 2016). The number of gene copies in this study is expressed as the ln number of gene copies (AOA, AOB, *nos*Z) per gram of soil dry weight.

2.5 CC-NCALC Model

The short-term expected N mineralization (or immobilization) stemming from CCs residues decomposition 1.5 months after their incorporation was assessed using a web-based model referred to as the 'Cover Crop N Calculator' (CC-NCALC). This model, developed by the University of Georgia (Woodruff et al., 2018; Thapa et al., 2022), as described in detail by Woodruff et al. (2018), computes an estimation of the total N released by incorporated CCs residues. It is an adapted and executed iteration of the N mineralization and immobilization subroutine extracted from the CERES-N model (Godwin and Jones, 1991). The model's functioning for the present investigation relies on the incorporation of diverse input parameters reported in detail by Raimondi et al. (2023a).

2.6 Statistical analysis

Descriptive statistics were computed for all the datasets to analyze the main feature of the data distribution and all the NFG abundances were transformed into the natural logarithmic form (ln), to reduce the skewness of the data.

Generalized linear models (glm) ('glm()' function in R software) were used to analyze how (i) the abundance of each nitrifier (AOA, AOB), denitrifier (*nirk, nosZ*), and fixing (*nifH*) gene; and (ii) the soil Nmin content and the estimated N released by the CCs residues (outcomes variables) were affected by the CCs treatment, the year, and the interaction between the two (prediction variables). Moreover, a glm was used to analyse how the total NFG abundance in each CC treatment (outcome variable), changed according to the NFG functional groups (AOA, AOB, *nirK*, *nosZ*, and *nifH*), the year, and the interaction between the two of them. The temporal patterns exhibited by the SWC variables were assessed employing a generalized least square (GLS) fitting technique adept at estimating the standard error considering the autocorrelation in the residual series (Cowpertwait et al., 2009; Campi et al., 2019). Models for repeated measures were constructed using the gls() function within the R software, using the CCs treatment as a fixed factor, whereas the SWC sampling dates as repetition factor. A Wald test Anova of each model (glm, and gls) was run and a post-hoc analysis (Sidak test for multiple sets of pairwise comparisons) was performed to estimate the marginal means with a 95% confidence interval. All the analyses were conducted for the three periods under analysis: WI, SP, SU.

Interactions among soil and crop parameters and NFGs abundance were explored using the principal components analysis (PCA () function in R software) for each period under analysis within each year. Based on the PCA analysis, the correlations among all the NFGs abundance, the soil parameters (SWC, and Nmin) and the CCs and/or weeds incorporated residues N release were investigated with the Spearman Rank Order Correlations test taking all the experimental years and treatments together.

3. Results

3.1 Meteorological conditions

Figure 1 shows the monthly average meteorological conditions and the irrigation events during the experimentation. The rainfall measured in the I, II, and III agronomic years - from CCs sowing until cash crop harvest - was 0.5%, 16.3%, and 35.3% lower than the 30-year average (820 mm).

From October to March (WI period) total rainfall was 380 mm in the I year, 279 in the II year, and 175 mm in the III year (30-year average = 378 mm). In the SP period spanning from April until the half of May, the I and III years registered a total mm of rain equal to -69.9% and -52.2% of that measured in the II year (113 mm) (30-year average = 114 mm). From the half of May until September (SU period), it was measured a total of 335 mm of rain in the I year, 193 mm in the II year, and 224 mm in the III year (30-year average = 326 mm).

In all three years, the hottest period was July -August (with an average of 24.1 °C in the I year, 23.7 °C in the II year, 25.9 °C in the III year), whereas the coldest was January (monthly average of 3.3 °C, 3.1 °C, and 3.0 °C in the I, II, and III year). In the long-term period (1992-2022), the average temperature of the hottest months (July- August) was 23.7 °C, whereas the average temperature of the coldest month (January) was 3.2 °C.

A similar pattern emerged for the soil temperature (first 0-40 cm layer) which had similar values across all three years, showing a maximum monthly average of 25 °C in July and a minimum of 4.6 °C in January. From October to March in all three years, an average of 8.8 °C was measured, whereas from April until May 15th the registered average was 15.3°C. From the half of May until September 22.6 °C were registered on average in all three years of experimentation.

The daily ET0 (Hargreaves equation modified by Berti et al. 2014) for all experimental years followed a similar trend with the lowest averages occurring from November to January (period average of 0.7 mm) and the highest values recorded from June to July (period average of 4.8 mm).

3.2 Soil N functional genes

All the NFGs abundances (number of ln gene copies per g soil^{-1}) are reported in Figure 2 and Figure 3. The posthoc results of the NFGs analysis are reported in Supplementary Table 1S for the upper soil layer, and Table 2S for the deeper soil layer.

3.2.1 WI period

The Δ AOA and Δ AOB abundances in the upper soil layer (Figure 2A) for the WI period were significantly different in the three years. A lower Δ AOA abundance was observed in the III year (-3.4 ln gene copies on average) compared to both the I and II years which showed similar counts (-1.1 ln gene copies on average). Differently, Δ AOB abundance was higher in the II year (3.7 ln gene copies) compared to the other two (-0.7 ln gene copies on average). A significant interaction CCs x year was found for both the Δ denitrifier genes (*nirK* and *nosZ*) and the Δ N fixation gene (*nifH*). The Δ *nirK* abundance was the highest in all the treatments of the II year (-0.06 ln gene copies) and the lowest in the NoCCs III year (-2.5 ln gene copies), with all the other treatments showing intermediate values with no significant differences. The Δ *nosZ* abundance had the highest abundance in the NoCCs I year (1.2 ln gene copies) and the lowest in the Succession II year (-4.3 ln gene copies). All the treatments in the I year and the Fixed II year showed no significant difference compared to the NoCCs I year in terms of Δ *nosZ* abundance. Similarly, there were no significant differences between all treatments in the III and the NoCCs II year compared to the Succession II year. The Δ *nifH* abundance was the highest in the Fixed II year (1.05 ln gene copies) and the lowest in the NoCCs III year (-3.2 ln gene copies), with all the other treatments showing intermediate values. The results of the comparative analysis among all the gene abundances in all the CC treatments and years are depicted in Table 3S and Table 4S and showed that both the highest (Δ AOB) and lowest (Δ *nosZ*) abundances were measured in the Succession II year, while all the others showed intermediate values.

In the deeper soil layer, (Figure 3A), Δ AOA, Δ *nifH* and Δ *nosZ* abundances had significant CCs treatment x year effect. Δ AOA abundance was the highest in the Succession II year (0.7 ln gene copies), whereas the lowest was in the Fixed I year (-2.2 ln gene copies). All other treatments showed intermediate values, except for the Δ AOA abundance in the Succession I year (-2.10 ln gene copies) which was lower than the highest value. Δ *nifH* abundance was the highest in the NoCCs II year (0.6 ln gene copies) and the lowest in both

NoCCs and Succession III year (-2.6 ln gene copies on average). All other treatments showed intermediate values. Succession II year (0.5 ln gene copies) was higher than the lowest values. Δ *nosZ* abundance was the highest in the Succession II year and the lowest in the Fixed III year (-2.1 ln gene copies), with all other treatments being intermediate. *nirK* did not show any significant difference, while Δ AOB abundance was only affected by the year. Specifically, it showed the highest abundance in the II year (3.3 ln gene copies) and the lowest in both the I and II years (1.5 ln gene copies).

3.2.2 SP period

The Δ AOA, Δ AOB, Δ *nosZ* and Δ *nifH* abundances showed no significant year or CC effects, in the upper soil layer (Figure 2B). The Δ *nirK* abundance was affected by the year, showing higher values in the I and III years (0.7 ln gene copies on average) than in the II one (0.4 ln gene copies). The comparative analysis among all the Δ gene abundances (Table 3S) did not show any significant difference. In the deeper soil layer (Figure 3B), a significant year x CCs interaction was found for all Δ NFG abundances. Specifically, among nitrifiers the Δ AOB abundance was the highest in the Fixed I year (1.4 ln gene copies) and the lowest in all treatments of III year and the Succession II year (1.2 ln gene copies on average). All other treatments did not show significant differences. Δ AOA abundance was highest in the Fixed I year (3.66 ln gene copies) and lowest in all treatments of the III year (-3.7 ln gene copies on average). All other treatments were intermediate, but all treatments of the II year differed from the Fixed I year, and the Succession I year differed from the lowest abundance. The denitrifiers Δ *nirK* and Δ *nosZ* abundances were the highest in the Fixed I year, showing 2.9 and 2.6 ln gene copies, respectively. The lowest Δ nirK abundance was found in all treatments of the III year (-2.5 ln gene copies on average), with all other treatments in-between. Similarly, Δ *nosZ* abundance was the lowest in all treatments of the III year (-2.4 ln gene copies on average), with all other treatments being intermediate. Δ *nifH* abundance was the highest (3.7 ln gene copies) in the Fixed I year, and the lowest in the Fixed III year (-2.1 ln gene copies). All other treatments were in-between with all values in the treatments of the I year differing from the lowest values and all data in the II year and in both NoCCs and Succession III year being different from the highest value. The comparative analysis among all the gene abundances (Table 4S) showed that all genes had the highest abundances in the Fixed I year and the lowest in both NoCCs I and II years, with all the others showing intermediate values.

3.2.3 SU period

The Δ AOB, Δ *nirK*, and Δ *nifH* abundances were significantly affected by the year, whereas the Δ AOA and Δ *nosZ* abundances were significantly affected by the CC x year interaction, in the upper soil layer (Figure 2C). Differently, in the deeper soil layer (Figure 3C), both nitrifiers, Δ *nosZ* and Δ *nifH* abundances were influenced by the year x CCs interaction, whereas Δ *nirK* abundance did not show any significant difference.

Δ AOB, Δ *nirK,* and Δ *nifH* abundances in the first 0-20 cm depth showed the same pattern with a higher abundance in the I and III years (0.3, 0.04, and 0.6 ln gene copies, respectively) showing a decrease in the II year. Δ AOA abundance was higher in the Fixed III year (1.94 ln gene copies) than the Δ NoCCs abundance of the II year (-1.1 ln gene copies). This last was also higher than the Δ AOA abundance measured in the NoCCs of the I (-1.8 ln gene copies). All other treatments showed intermediate values between Fixed III year and NoCCs II year. The highest Δ *nosZ* abundance was registered in both Fixed and Succession at III year (averaging 0.84 ln gene copies), followed by Fixed II year (-1.7 ln gene copies) and the lowest NoCCs I year (-2.4 ln gene copies). All other treatments exhibited intermediate values. The comparative analysis among all the gene abundances (Table 3S) showed the highest values for Δ AOA in the Fixed and Succession III year, and the lowest for the Δ *nosZ* in the NoCCs I year.

In the 20-40 cm depth, all NFG showed the lowest abundance in the NoCCs I year. Δ AOB abundance was the highest in both NoCCs and Succession II year (0.8 ln gene copies on average), and the lowest in the NoCCs I year (-0.95 ln gene copies), with all other treatments being intermediate. Δ AOA abundance was highest in all treatments of III year and Succession II (1.3 ln gene copies on average), and lowest in NoCCs I (-2.01 ln gene copies) with intermediate values measured in all other treatments. The denitrifier Δ *nosZ* abundance was highest in both Succession II and Fixed III (1.3 ln gene copies on average) and lowest in NoCCs I year (-1.4 ln gene copies). All other treatments were in-between. The Δ *nifH* abundance showed the highest values in the Fixed III year (2.4 ln gene copies) and the lowest in the NoCCs I year (-2.2 ln gene copies). All other treatments were intermediate with the NoCCs III year and the Succession II and III years being different from the lowest values, and the the NoCCs I year being different from the highest values. The comparative analysis among all the gene abundances (Table 4S) showed that the total Δ abundance of the NFG highest in the Fixed III year and the Succession II year, followed by the Fixed II year, Succession I year and Fixed I year. However, the lowest Δ abundance was found in the NoCCs I year.

3.3 Soil and crop parameters

3.3.1 Soil water content

The Δ SWC values are reported in Figure 4, whereas the results of the post-hoc analysis are depicted in Table 5S -14S. The Δ SWC showed significant variable trends in time in all experimental years, during the winter period from October until March, early spring from April to May, and late spring-summer period from May to September. No significant differences were observed among treatments.

The trends of water accumulation and reduction in the soil were similar for the 0-20 and 20-40 depths, despite the absolute values being different. In the upper soil layer, the Δ SWC followed an increasing trend from October until February 2021 when it reached + 24.1 mm (February 11th), and from October until January 2022 when it reached +15.5 mm (January 12th). After these highest values being reached, a decreasing trend was registered in both years until the end of March (+8.1 mm March 25th 2021; + 10 mm March 23rd 2022). A similar rising Δ SWC trend was observed in the 20-40 cm depth from October reaching the highest values of +31.4 mm in February 2021 (February 11th), and of +15.2 mm in January 2022 (January 12th). A downward trend was then observed until March when +14.9 mm were registered in 2021 (March 25th) and -2.7 mm in 2022 (March 23rd).

During the cash crop growing seasons, from April-May until September a descending Δ SWC trend was observed for both depths (0-20 cm and 20-40 cm). September was the month with the lowest Δ SWC in all experimental years, measuring: i) in the upper soil layer -0.84 mm in the I year, -9.3 mm in the II year, and -13.2 mm in the III year; and ii) in the deeper soil layer, -9.6 mm in the I year, -30.9 in the II year, and -25.9 mm in the III year.

3.3.2 Soil Nitrate (Nmin)

Soil Δ Nmin contents are reported in Figure 5. In the WI period a significant CCs and year interaction was found in both the upper and deeper soil layers. In the CC Succession II and III year and NoCCs III year the Δ Nmin content was close to 0 (+0.3 kg ha^{-1} on average) and showed negative values in the other treatments, reaching the lowest value in the NoCCs I year (-41.2 kg ha^{-1}).

Both Fixed and Succession in the I year showed values that didn't significantly differ from Fixed II year and NoCCs I year, whereas Fixed III year didn't show significant differences between the highest values and Fixed II year. A similar result was observed for the soil Δ Nmin at the 20-40 cm depth, where the highest values were recorded in the Succession and NoCCs II year (+10.6 kg ha^{-1} on average) and the lowest in all treatments of the I year and the Fixed of the III year (averaging -26.5 kg ha^{-1}). Other treatments exhibited values falling in the intermediate range.

Differently, for the SU period, CCs had no significant effect in the upper soil layer. In the deeper soil layer, the soil Δ Nmin was significantly higher in the III year (+23.4 kg ha^{-1}) than in the other two (-5.4 kg ha^{-1}).

3.3.3 Short-term CCs biomass N release

The estimated N released by the CCs residues 1.5 months after their termination was significantly influenced by the interaction between CCs management and experimental year (Table 1). The highest values were observed in both the NoCCs and Succession of II (clover) and III (mustard) years (13.4 kg ha^{-1}), followed by Fixed II year (4.5 kg ha^{-1}). Triticale residues in the Fixed III year reported the lowest N release value (-1.7 kg ha^{-1}), whereas all the treatments of the I year had intermediate values (1.2 kg ha^{-1}) between Fixed II and III year.

3.3.4 Cash crop yield

The cash crops yield was not significantly affected by the CCs treatment. On average, the silage maize produced a dry biomass of 17.9±1.2 Mg ha^{-1} in the I year and 15.4±1.1 Mg ha^{-1} in the II year (Raimondi et al., 2023b). The average soybean grain yield (III year) was 3.1±0.1 Mg ha^{-1}.

3.4 Variability of soil, crop parameters, and soil NFGs in CC treatments

The Euclidean space obtained with the PCA was built to represent the maximal dataset variability along the two axes: it illustrates how the variability of the three CCs treatments is explained by the different variables such as CCs production yield and N uptake, SWC, soil Nmin and all NFG in the upper soil layer. The PCA performed for the deeper soil layer did not show any significant clusterization. For this reason, only the results of the shallow layer are presented (Figure 6).

The results of the PCA analysis for the WI period showed that the first two principal components (PCs) explained 75.3%, 60.8%, and 57.2% of the dataset variability of the corresponding I, II, and III years. In the I year, the two CCs treatments overlapped and clustered along with the PC1 and PC2 separately from the NoCCs treatment. The eigenvalue loadings for the PC1 were dominated by all NFG, indicating a strong positive correlation among all of them, which primarily accounted for the dataset variability of the Fixed and Succession treatments. On the other hand, the PC2 was dominated by the soil Nmin. The dataset of the NoCCs treatment was mostly explained by a negative correlation of PC2 with Nmin. In the II year, the Succession and NoCCs treatment overlapped, whereas the Fixed treatment formed a distinct cluster that partially overlapped with adjacent areas. The eigenvalue loadings for the PC1 were primarily influenced by all NFG and Nmin. A positive correlation among all of the NFGs explained the dataset of all three CC treatments. Conversely, the negative correlation between PC1 and Nmin only explained the dataset of the Succession and the NoCCs treatment. The PC2 was dominated by SWC. The dataset of the Fixed treatment was mostly characterized by a negative correlation of PC2 and SWC. In the III year, there was a substantial overlap among all three CCs treatments. The eigenvalue loadings for the PC1 were dominated by all NFGs. A positive correlation among all NFGs explained the dataset of all CC treatments. The PC2 instead was dominated by Nmin and SWC. A negative correlation between PC2, soil Nmin and SWC mostly described both Succession and Fixed treatment.

The results of the PCA analysis for the SP period showed that the first two principal components (PCs) explained 76.8%, 77.3%, and 74.4% of the dataset variability of the corresponding I, II, and III years. In the I year, the three CCs treatments formed separated clusters. The eigenvalue loadings for the PC1 were dominated by all NFG and CCs N release. A strong positive correlation among all of NFGs mostly explained the dataset of the NoCCs, whereas a negative correlation between PC1 and CCs N release mostly described the dataset of the Succession treatment. Conversely, the Fixed treatment was described by the absence of correlation among all variables. The PC2 was dominated by CCs N release. In the II year, all treatments overlapped. The eigenvalue loadings for the PC1 were dominated by AOB and *nosZ* as well as *nirK* and *nifH*. The positive correlation between *nirK* and *nifH* and both of them with PC1 mostly described the Succession and NoCCs treatments. The PC2 was dominated by CCs N release and AOA. A negative correlation between PC2 and AOA mostly described the dataset of the Fixed treatment, whereas the positive correlation between CCs N release and PC2 described the Succession and the NoCCs treatments. In the III year, both Succession and NoCCs treatments overlapped, whereas the Fixed treatment formed a separate cluster that partially overlapped with the adjacent areas. The PC1 was dominated by all NFG. A positive correlation between all of them and the PC1 mostly described the dataset variability of the Succession and the NoCCs treatments, while the positive correlation between AOA and *nosZ* with PC1 described the Fixed treatment. The PC2 was dominated by CCs N release whose positive correlation mostly described the Succession and the NoCCs treatments.

The results of the PCA analysis for the SU period showed that the first two principal components (PCs) explained 62.7%, 60.7%, and 62.5% of the dataset variability of the I, II, and III years respectively. In the I year, both Succession and Fixed treatments overlapped, with the Succession treatment clustering separately with an overlapping area. The PC1 was mostly dominated by all NFGs and Nmin. The positive correlation among all NFGs mostly described the dataset variability of the Fixed and the Succession treatments, while the negative correlation of Nmin with PC1 mostly described the dataset variability of the NoCCs. The dataset variability of both Succession and Fixed treatments were mostly described by the negative correlation between PC2 and SWC. In the II year, all treatments overlapped as in the III year. In the former, the PC1 was dominated by all NFGs, Nmin, and

SWC, whereas the PC2 was dominated by SWC and Nmin. In the III year, the PC1 was dominated by all NFGs and Nmin, whereas the PC2 was dominated by SWC.

3.5 Relationship between soil parameters, CCs biomass N release, and NFGs

In the upper soil layer, all the NFGs were positively correlated with each other (Table 2). No significant correlation was found between NFGs and the CCs residues N release. SWC, negatively correlated with AOA (-0.3), whereas correlated positively with *nosZ* (+0.4). Furthermore, a positive correlation was detected between soil Nmin and *nosZ* (+0.2), as well as AOA (+0.1). Differently, *nifH* negatively correlated with soil Nmin (-0.6).

In the deeper soil layer, all the NFGs were positively correlated with each other (Table 3), as in the upper soil layer. CCs residues N release positively correlated with AOB (+0.2), but negatively with *nifH* (-0.2). Negative correlations were found between SWC and AOA (-0.1), AOB (-0.2), and *nifH* (-0.1). Conversely, both *nosZ* (+0.3) and *nirK* (+0.2) were positively correlated with SWC. AOA was the only NFG showing a significant positive correlation with soil Nmin (+0.1).

4. Discussion

4.1 CCs effect on cash crop production

The absence of CC effects on cash crop yields in the initial years following their introduction holds substantial importance. The potential negative impact of winter CCs on the ultimate yield of cash crops is a critical factor that may affect farmers' decisions regarding the adoption of CCs (Singer et al., 2007). Our findings align with earlier research by Marcillo and Miguez (2017), who demonstrated that grasses CCs do not induce alterations (either increases or decreases) in maize yield compared to fallow soil. Notably, studies such as Krueger et al. (2011) have indicated that delaying the termination of rye CC in spring by four weeks rather than an early termination can negatively affect maize yield due to potential N immobilization subsequent to termination (Hunter et al., 2021) and resulting delays in maize planting. The timely spring termination of CCs (at the end of March in our study site) likely averted maize yield reduction following the rye CC. This temporal gap might have allowed for adequate residue decomposition (Hashemi et al., 2013) and potentially minimized allelopathic impacts from the rye species (Kelton et al., 2012).

The observed similar maize yields following the clover (Succession), triticale (Fixed), and weeds (NoCCs) treatments in the II year could potentially be attributed to the N fertilization applied to the maize crop, as supported by earlier investigations (Miguez and Bollero, 2006; Marcillo and Miguez, 2017). The application of 200 kg N ha^{-1}, as carried out in our experiment, might indeed hinder the capacity of leguminous CC to enhance maize yield during the initial years after their introduction. Similarly to maize, the soybean yield was not affected by the presence of triticale (Fixed) or mustard (Succession) CCs in the III year. Indeed, despite the species, also Tavares-Silva et al. (2012) noted that winter CCs showed no effect on soybean yield. Acuña et al. (2014) in the Midwest USA observed no different soybean yield after triticale CC compared to the control plots without CC. On the contrary, Calonego et al. (2010), observed an augmented soybean yield after triticale CC. This enhancement was attributed to better soil structuring following triticale as opposed to the control plots without CC. All findings must be interpreted within the context of comparable weed biomass in both NoCCs II and III years, with that of CCs treatments. Therefore, our evaluation did not contrast the presence of CCs with bare soil, but rather compared CCs with spontaneous vegetation cover. Both types of cover represented vegetative components consuming water and uptaking nutrient resources, demonstrating potential interactions with subsequent cash crops, that might not have been observed in the case of a bare soil condition.

4.2 Cover crops seasonal effect on SWC and N cycle

4.2.1 WI period

Across all years, SWC exhibited a consistent declining trend in the months preceding the CCs termination across all treatments. This trend corresponded to the resumption of plant growth, coinciding with the increase in air and soil temperatures. Upon the termination of CCs, SWC levels in plots treated with CCs did not exhibit any significant disparity compared to plots without CCs. This observation indicates that no competitive effect on water availability at cash crops sowing was exerted by CCs compared to the NoCCs treatment, contrasting with findings in previous studies (Alonso-Ayuso et al., 2014, 2018; Gabriel et al., 2019). Our results might be justified by the similar biomass production observed between CCs and weeds in the treatment without CCs, particularly evident in the second and third years of the study.

Considering the N cycle, during the I year of investigation (equivalent to the first year of CCs introduction in the experimental site), the soil Nmin levels registered at CCs sowing (Raimondi et al., 2023b) diminished by the CCs termination time, consistently in all treatments. However, while the higher grasses N uptake might be related to the decreased Nmin content in both Fixed and Succession treatments (catch crops) (Ruffo and Bollero, 2003), the minimal weeds biomass production and N uptake revealed a higher potential for N loss in the environment in the NoCCs. The initial Nmin content might have fostered the potential complete denitrification process in the shallow soil layer of all treatments. A high Nmin substrate is a key promoting factor of denitrification activity fostering the abundance of both *nosZ* and *nirK* (Waghmode et al., 2018; Zhou et al., 2011), along with high SWC, often associated with increased rainfall (van Spanning et al., 2007). In the I year, higher rainfalls were registered compared to the subsequent experimental years. Such rainfalls might have limited the potential nitrification activity (AOB), as it was reported that AOB tends to be hindered by elevated precipitation levels (Liu et al., 2017). In the II year, a substantial potential N fixation activity (*nifH*) was measured in the upper layer of the Fixed treatment (triticale) where lower values of Nmin were measured compared to other treatments. This result was in accordance with a previous study by Ikeda et al. (2014) and strengthened by the significant negative correlation found between *nifH* and Nmin. Differently, an elevated potential nitrification activity (AOA) was measured in the deeper layer of Succession treatment (clover) where the highest Nmin content was recorded (confirmed by their significant positive correlation), in compliance with the findings of Momesso et al. (2022). In the III year, similar residual soil Nmin was registered in all treatments (all residues around zero), but the lowest was registered in the deeper soil layer of the Fixed treatment. This lowest value combined with the general low SWC and rainfall, might have contributed to the lowest abundance of *nosZ* denitrifier measured in the Fixed III year. This inference is consistent with the suggestions of Linton et al. (2020).

4.2.2 SP period

In our study, the use of the CC-NCALC model, detailed by Woodruff et al. (2018) and Thapa et al. (2022), unveiled an overall net mineralization of all CCs and weeds residues, except for triticale in the Fixed treatment of the III year which show an estimated net immobilization. These findings confirmed previous observations on triticale reported by Rosolem et al. (2018) and White et al. (2016). Nevertheless, no significant difference was observed in terms of genes responsible for nitrification, denitrification, or N fixation activities in the shallow soil layer among different CC treatments in all three experimental years. These findings stand in contrast to numerous preceding investigations that have emphasized the significant influence of incorporating CC residues on alterations in soil micro-fauna activity (McDaniel et al., 2014; Ouyang et al., 2018). In our study, these observed discrepancies could potentially be attributed to the tillage operation carried out for the CCs termination (shredding with a rotary mulcher), and the subsoil tillage performed at 30 cm depth followed by the rolling harrow for the cash crop seedbed preparation. These tillage operations might have affected the soil microbial community concealing potential differences in soil NFGs in the upper 0-20 cm soil depth, as already observed by Smith et al. (2016). Likewise, the pre-sowing N fertilization (32 kg N ha^{-1}) applied 30 (in the I year) and 15 (in the II year) days before the soil samples collection, might have impaired the soil N-cycling communities as previously reported by Kim et al. (2022b). 1.5 months after CCs termination, the N released by both grasses CCs (triticale and rye) in the I year, showed low values considering all treatments of all years, not different from the lowest measured for triticale in the III year. These results confirmed that both rye and triticale tend to exhibit a slow N release due to their composition (C:N ratio), as already widely observed in prior research studies (Blanco-Canqui et al., 2015; Quemada and Cabrera, 1995). In addition, in the first weeks after sowing (I year), a lower maize growth was observed in both Fixed (triticale) and Succession (rye) treatments (previous survey conducted in the same experimental site by Raimondi et al., 2023b). The high residues N release estimated for the clover species aligned with various previous studies (Coombs et al. 2017; Yang et al. 2020), and it is mostly attributed to the residue quality with low C:N ratio. Nonetheless, the observation of the present study warrants further consideration of the importance of investigating the spontaneous weeds' role in the N dynamics of an agroecosystem, as suggested by Li et al. (2020).

Despite the observed differences within the three years, higher levels of Nmin and N released were registered by all treatments in the II year compared to the I one. This might have discouraged potential N fixation activity (*nifH* abundance), as previously reported (Bao et al., 2014), in all treatments of II year compared to the grasses CCs species of the I year (especially triticale).

Mustard CC (Succession III year) showed an intermediate behavior between the grasses and legume CCs species cultivated throughout the experimental period, as it showed an N uptake similar to grasses with a faster decomposition rate comparable to that of clover, as earlier reported in research studies (Collins et al., 2007; Finney et al., 2016). However, the mustard residue N release measured in the SP period might be also related with the observed frost winterkill of mustard described by Raimondi et al. (2023a). This phenomenon is consistent with the findings of Weinert et al. (2002), who indicated that CCs experiencing winterkill tend to release and leach N more rapidly than those that survive the winter. Despite the higher residues N release measured for mustard and weeds compared to triticale (N immobilization), the ensuing soybean crop did not show any different growth pattern among treatments.

Interestingly, no distinctions were evident in terms of potential soil N transformation processes during the III year. All NFGs exhibited the lowest abundance. These results might relate to the minimal rainfall measured in the SP phase of III year. Prior studies have demonstrated that reduced rainfall can induce alterations in soil microbial compositions and enzyme activities, consequently leading to a reduction in processes such as soil N mineralization, nitrification, and denitrification (Hartmann et al., 2013; Homyak et al., 2017). This influence is particularly pronounced in humid regions, similar to the environmental context of the current study (Wu et al., 2022).

4.2.3 SU period

Consistent decreasing SWC trends were observed from cash crop sowing until harvesting in all treatments. Aligned with the result of WI period, the presence of CCs during the winter season did not exhibit any discernible impact on SWC during the subsequent growth period of cash crops, compared to the control treatment.

Analyzing the N dynamics, in the I year, it has been observed that AOA and *nosZ* abundance at 0-20 cm depth in the NoCCs showed the lowest abundances compared to all treatments in all experimental years. This might have been related to both the lower rainfall registered in this period compared to the other years and to the low amount of weed residues incorporated compared to that of both the CC treatments. These observations align with previous studies (Cheng et al. 2014; Hu et al. 2023b). The lower *nosZ* abundance heightened the risk of N_2O emission in the NoCCs treatment confirming the observation of Behnke et al. (2022) which observed a lower denitrifier abundance where no CCs were cultivated. Furthermore, across the deeper soil layer, all NFGs demonstrated the lowest abundances in the NoCCs treatment in the I year compared to all other treatments. These patterns imply that the presence of CC residue led to an expanded pool of readily available N, thereby fostering all N transformation processes in comparison with the fallow treatment with negligible weeds presence. In the II year, within the upper soil layer, negligible differences were also evident in terms of NFGs. AOB, *nirK,* and nifH in this II year exhibited lower abundance in comparison to both the III year, when soybean potentially facilitated a generally higher NFGs abundance, as suggested by Behnke et al. (2022). In contrast, within the 20-40 cm soil layer, the most pronounced potential for nitrification (as indicated by AOA and AOB gene abundance) and denitrification (represented by *nosZ* gene abundance) activities were observed after clover, in consonance with earlier findings (Momesso et al., 2022). In the III year of the study the presence of soybean in both soil layers resulted in notably higher residual Nmin across all treatments compared to preceding years. Nevertheless, in both soil depths, AOA and *nosZ* demonstrated the highest abundance only in the CCs treatment (with triticale and mustard). This pattern suggests that the biological N_2 fixation of soybean could potentially enhance nitrification and denitrification activity in the presence of residues with high C:N ratio (>30) (see Raimondi et al., 2023b). This could be attributed to a higher N_2 fixation by soybeans due to an increase in nodule mass stimulated by the elevated C:N residues ratio, as indicated by Kihara et al. (2011). This finding highlights the intricate interplay of biological processes that impact nitrogen dynamics in the soil, aligning with insights from prior studies (Norton and Ouyang, 2019; Paustian et al., 2016).

It is important to interpret these results concerning NFGs within the context of a three-year CC implementation, recognizing that this timeframe might not suffice to instigate substantial alterations in the N cycling communities. This initial understanding offers valuable insights into the capabilities and constraints of implementing CCs within established agricultural systems, a perspective underscored by Kim et al. (2022a). Consequently, future research into these aspects will be needed over a more extended temporal horizon, seeking to comprehensively assess the long-term implications and dynamics associated with these processes.

5. Conclusions

It holds significance to contextualize the outcomes presented in this study within the framework of the initial years following the introduction of the CCs. CC presence and species management did not exhibit a significant influence on the yield of maize and soybean Nonetheless, the study affirms that CCs and cash crop species, as well as the seasonality of crop sequence and management operations, are critical factors shaping the soil N cycling dynamics, intricately governed by N cycling microbial communities, and the temporal variation of SWC.

Grass CCs can reduce soil Nmin levels (catch crops) and stimulate microbial N-fixation activity (*nifH*) prior to cash crop sowing. They potentially increase the risk of N_2O production (lower *nosZ*) at their termination time when low rainfall occurs during the winter season. Clover, maintaining the highest Nmin level upon CCs termination, enhances potential microbial nitrification. The absence of CC without weed control doubles weed presence within a year risking of increase winter seedbanks in the short term.

Agronomic operations (e.g., CCs termination, seedbed preparation, N fertilization) may disrupt potential differences in N transformation processes due to CCs residue incorporation. Nevertheless, by cash crop harvest time, all CCs residues promote increased microbial-mediated nitrification and denitrification activity compared to weed-free bare soil. Notably, when cultivating soybeans, NFGs abundance is significantly higher than with maize, underscoring the role of cash crop species in shaping N transformation dynamics. Clover, mustard, and weed residues when incorporated in the soil promptly release substantial N, while grasses may decompose more slowly, potentially causing N immobilization (e.g., triticale) which can persist throughout the cash crop growing season.

Soil Nmin and NFGs are sensitive indicators for assessing chemical and biologically mediated N cycling dynamics affected by agricultural practices. However, their sensitivity can be influenced by various factors, making it challenging to distinguish the main effect under analysis. Our findings recommend soil sampling at CCs' termination before seedbed preparation to assess their impact on N dynamics through NFGs and soil Nmin indicators. So, to utilize these indicators effectively, careful consideration must be given to the timing of sample collection within the crop rotation.

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Figures

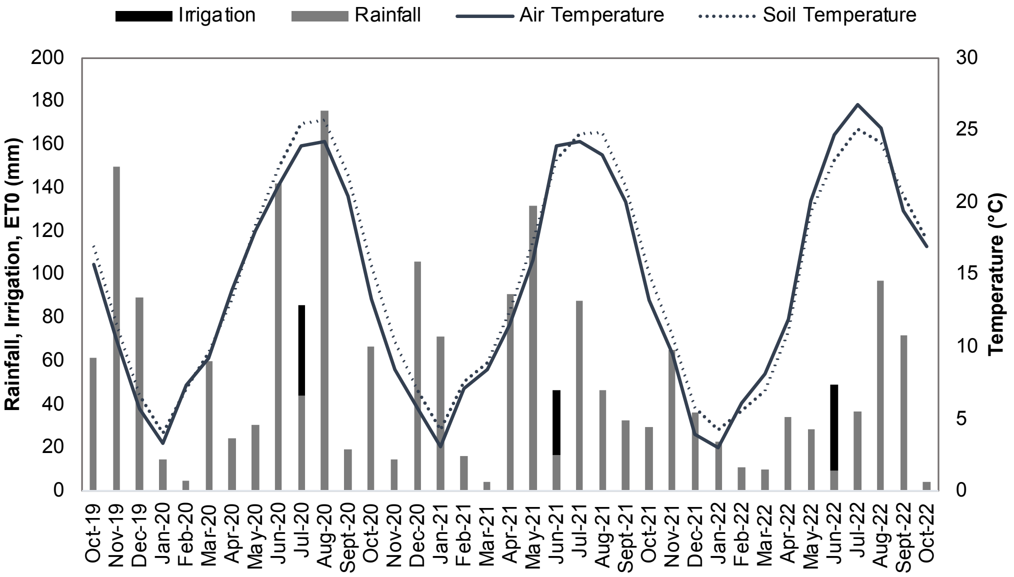

Figure 1. Monthly rainfall (mm), irrigation (mm) and mean air and soil temperature (°C) during the experimental period (2019-2022) at “L. Toniolo” experimental farm (Padova, Italy).

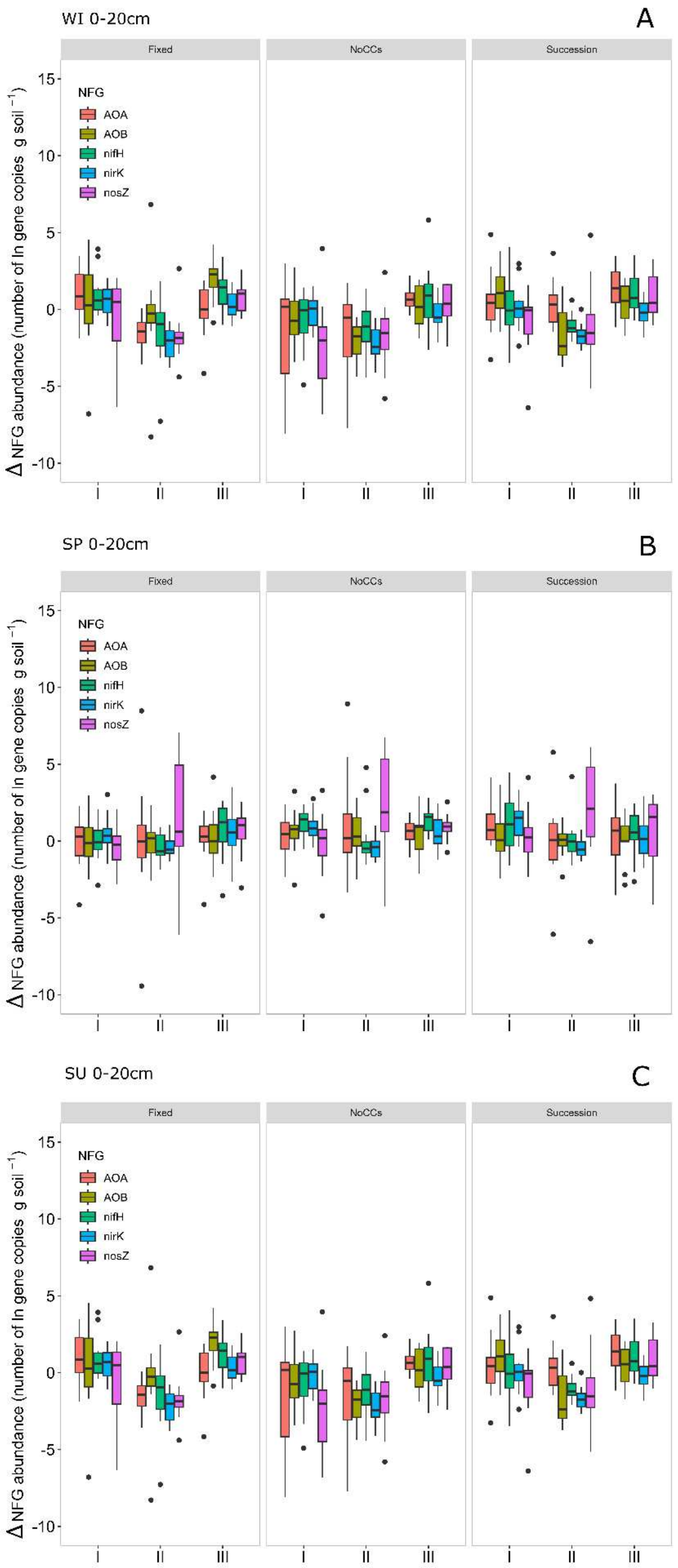

Figure 2. Δ ln of soil nitrogen functional genes abundances (Ammonia-oxidizing archaea, AOA *amoA*; ln Ammonia-oxidizing bacteria, AOB *amoA*; *nosZ*; *nirK*; *nifH*) measured at 0-20 cm soil depth for each period analyzed (cover crop growing season- WI - A; first 1.5 months after cover crop termination- SP - B; cash crop growing season- SU - C) in each

cover crop treatment (Fixed; Succession; No cover crop) and year of experimentation (I, II, III).

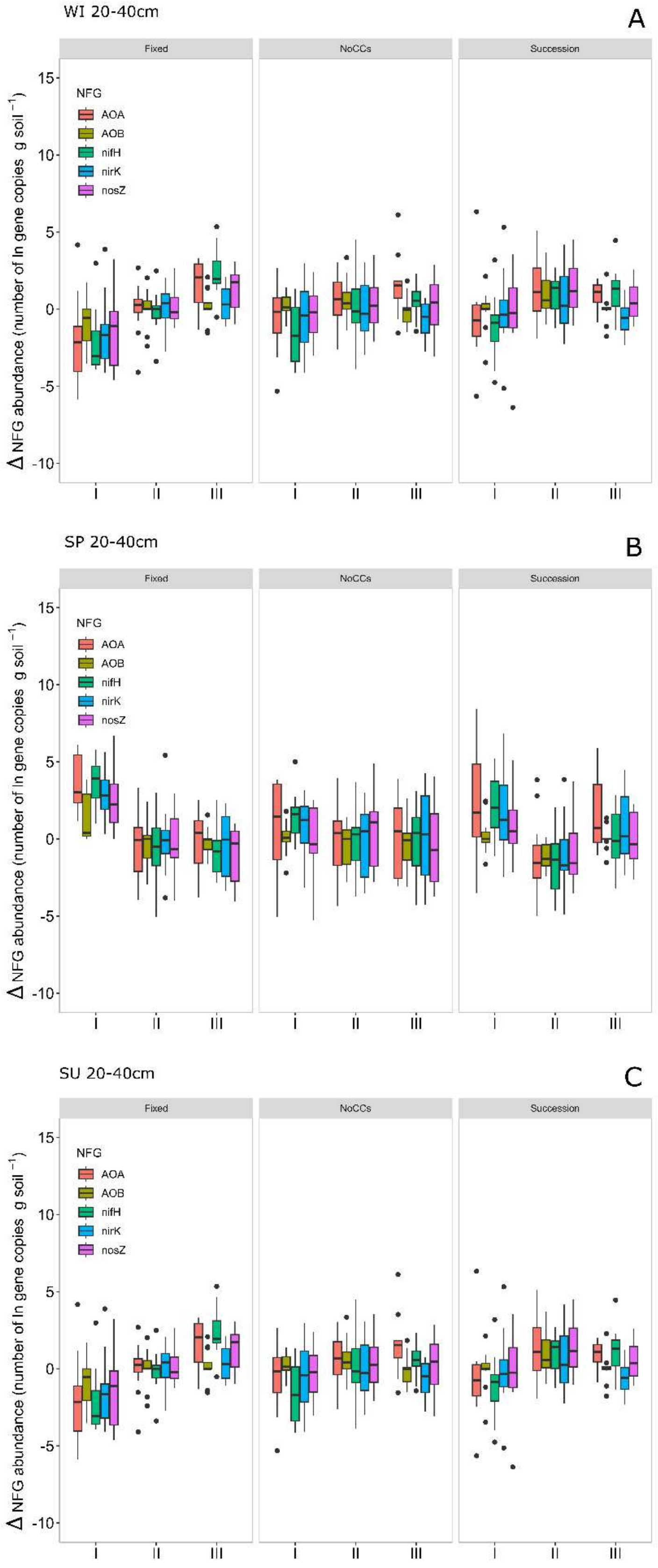

Figure 3. ln of soil nitrogen functional genes abundances (Ammonia-oxidizing archaea, AOA *amoA*; ln Ammonia-oxidizing bacteria, AOB *amoA*; *nosZ*; *nirK*; *nifH*) measured at 20-

40 cm soil depth for each period analyzed (cover crop growing season- WI - A; first 1.5 months after cover crop termination- SP - B; cash crop growing season- SU - C) in each cover crop treatment (Fixed; Succession; No cover crop) and year of experimentation (I, II, III).

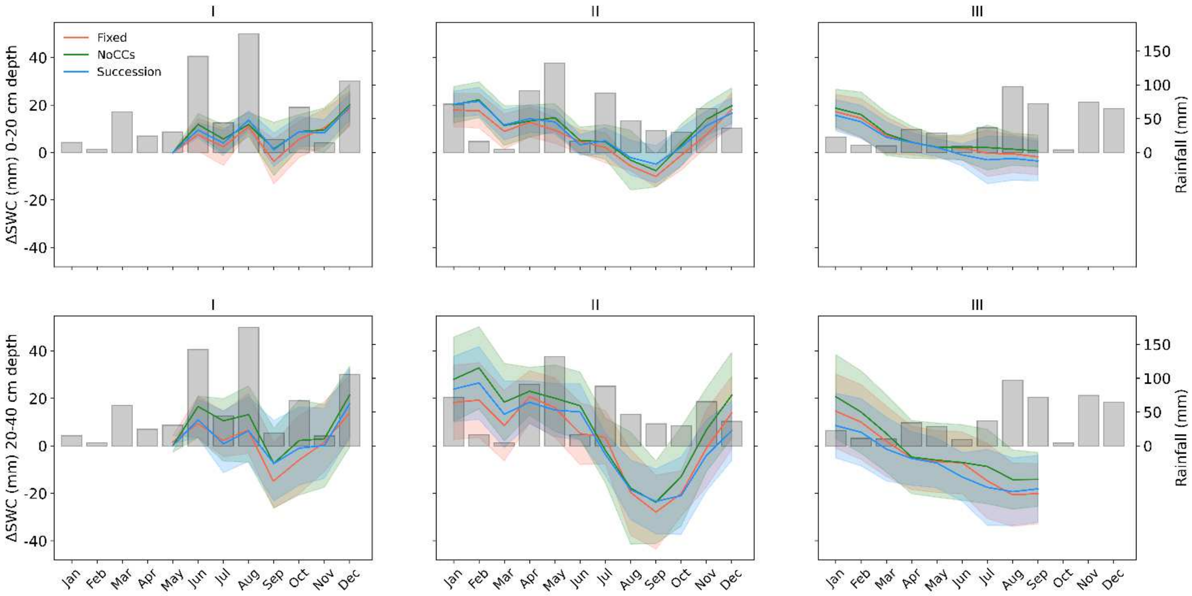

Figure 4. Δ Soil water content (SWC) (mm) in the 0-20 and 20-40 cm layers over the three experimental years (I, II, III) for the three cover crops treatments (Fixed: triticale. Succession: rye in 2020, clover in 2021, mustard in 2022. NoCCs: absence of cover crops). The ribbon around the lines represents the standard error. The monthly rainfalls (mm) are reported as grey bars in the plot.

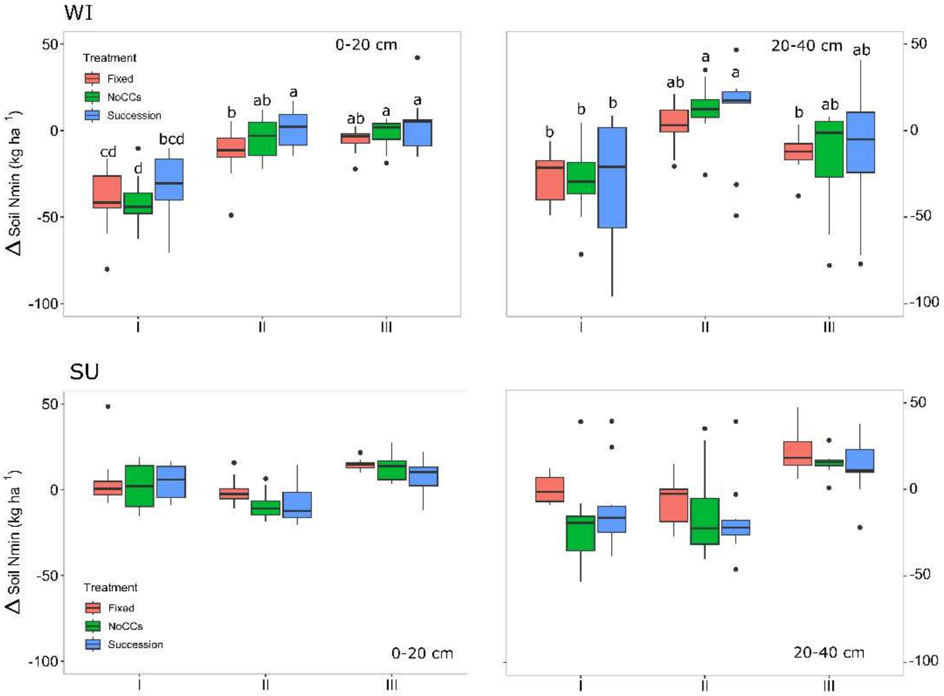

Figure 5. Average Δ soil Nmin (NO_3^- - N) (kg ha^{-1}) measured at 0-20 and 20-40 cm depths for two periods under analysis (cover crop growing season- WI; cash crop growing season-

SU) in each experimental year (I, II, III). Different lowercase letters indicate significant differences among cover crops treatments and years—significance (p value ≤ 0.01) obtained with Sidak post hoc test.

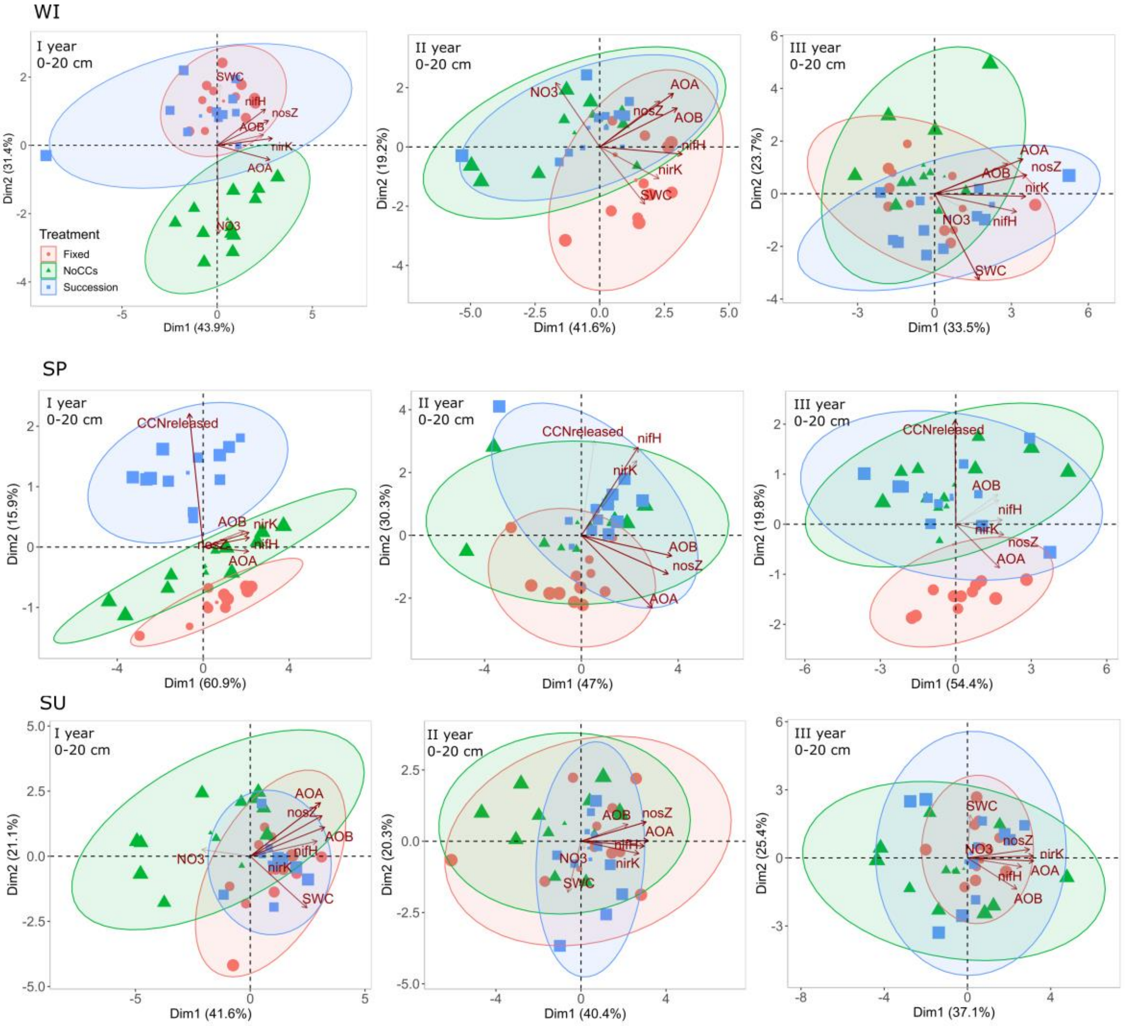

Figure 6. Principal Component Analyses of the soil N functional genes (ln Ammonia-oxidizing archaea, AOA *amoA*; ln Ammonia-oxidizing bacteria, AOB *amoA*; ln *nosZ*, *nosZ*; ln *nirK*, *nirK*; ln *nifH*, *nifH*), crop parameters (cover crops and/or weeds residues N release) and soil parameters (soil water content and soil NO_3^-content - NO3) measured at 0-20 cm soil depth in each cover crop treatment and period under analysis (cover crop growing season- WI; first 1.5 months after cover crop termination- SP; cash crop growing season- SU) of each experimental year (I, II, III). Vectors show the dominant significant components driving the separation of the three cover crop treatments.

Tables

Table 1. Cumulative N released by cover crops 1.5 months after their termination (NRT) estimated by the predictive model CC-NCALC. Fixed: triticale. Succession: rye in 2020, clover in 2021, mustard in 2022. NoCCs: absence of cover crops. * Significance (p value <0.001); ns = not significant (Wald test ANOVA). Different letters indicate significant differences.

Year	**Treatment**	**NRT (kg ha^{-1})**	
I	NoCCs	0.9 ± 0.02	bc
	Succession	2.6 ± 0.09	bc
	Fixed	0.2 ± 0.01	bc
II	NoCCs	13.6 ± 0.2	a
	Succession	14.2 ± 0.2	a
	Fixed	4.5 ± 0.1	b
III	NoCCs	15.2 ± 4.2	a
	Succession	10.5 ± 3.6	a
	Fixed	-1.7 ± 0.3	c
Treatment		ns	
Year		ns	
Treatment x Year		*	

Table 2. Spearmen correlation coefficient for soil parameters at 0-20 cm depth (SWC: soil water content; Nmin: soil mineral nitrogen -NO_3^-; soil N functional genes – nitrifiers, denitrifiers, fixator) and crop parameter (*cover crops and/or weeds biomass N release) in all the three years of experiment in all the CC treatments. Bold: significance at $p<0.05$.

	Nitrifiers		Denitrifiers		Fixator	Soil parameters		Biomass
	AOB *amoA*	**AOA *amoA***	***nirK***	***nosZ***	***nifH***	**SWC**	**Nmin**	**Residues* N release**
AOB *amoA*		**0.75**	**0.76**	**0.75**	**0.69**	-0.12	0.01	0.073
AOA *amoA*			**0.76**	**0.79**	**0.79**	**-0.30**	**0.12**	0.16
nirK				**0.71**	**0.83**	0.17	0.09	0.03
nosZ					**0.69**	**0.39**	**0.24**	-
nifH						-0.21	**-0.57**	0.11

Table 3. Spearmen correlation coefficient for soil parameters at 20-40 cm depth (SWC: soil water content; Nmin: soil mineral nitrogen -NO_3^-; soil N functional genes – nitrifiers, denitrifiers, fixator) and crop parameter (*cover crops and/or weeds biomass N release) in all the three years of experiment in all the CC treatments. Bold: significance at $p<0.05$.

	Nitrifiers		Denitrifiers		Fixator	Soil parameters		Biomass
	AOB *amoA*	**AOA *amoA***	***nirK***	***nosZ***	***nifH***	**SWC**	**Nmin**	**Residues* N release**
AOB *amoA*		**0.57**	**0.57**	**0.57**	**0.52**	**-0.13**	0.008	**0.25**
AOA *amoA*			**0.86**	**0.80**	**0.76**	**-0.21**	**0.14**	0.15
nirK				**0.81**	**0.72**	**0.17**	0.06	0.12
nosZ					**0.72**	**0.26**	0.14	0.15
nifH						**-0.13**	-0.15	**-0.22**

Supplementary materials

Table 1S. Average genes abundance (AOB, AOA, nirkK, nifH, nosZ) in the 0-20 cm soil layer (number of ln gene copies per g soil^{-1}) for each period analysed (cover crop growing season- WI; first 1.5 months after cover crop termination- SP; cash crop growing season- SU), in each year and cover crops treatments. Different letters indicate significant differences (Wald test ANOVA); ns = not significant.

Period	**Year**	**Treatment**	**Genes abundance in the 0-20 cm depth (number of ln gene copies per g soil^{-1})**									
			AOB		**AOA**		**nirK**		**nifH**		**nosZ**	
WI	**2020**	**Fixed**	-0.55	B	-1.39	A	-0.64	abc	-0.80	bcd	0.21	abc
		NoCCs	0.02		0.82		-0.41	ab	-0.15	ab	1.22	a
		Succession	-0.11		-0.47		-0.82	abc	-1.07	bcd	0.91	ab
	2021	**Fixed**	3.39	A	-1.31	A	-0.09	a	1.05	a	-0.53	ab
		NoCCs	2.88		-2.48		0.02	a	-0.34	bc	-2.10	bcd
		Succession	4.96		-1.99		-0.11	a	-0.45	bc	-4.26	d
	2022	**Fixed**	-1.22	B	-3.34	B	-2.26	bc	-2.63	cd	-2.35	bc
		NoCCs	-1.52		-3.31		-2.48	c	-3.21	d	-2.92	cd
		Succession	-1.01		-3.59		-1.65	abc	-2.50	bcd	-2.56	cd
	Treatment		ns		ns		ns		ns		ns	
	Time		$p<0.001$		$p<0.001$		ns		ns		ns	
	Treatment x Time		ns		ns		$p<0.001$		$p<0.001$		$p<0.001$	
SP	**2020**	**Fixed**	-0.14		-0.07		0.5	A	-0.06		-0.4	
		NoCCs	0.57		0.38		0.98		1.19		-0.16	
		Succession	0.36		1.13		1.34		0.15		0.34	
	2021	**Fixed**	-0.1		-0.03		-0.4	B	-0.44		1.37	
		NoCCs	0.34		1.4		-0.42		0.19		2.25	
		Succession	0.04		-0.04		-0.39		0.12		1.98	
	2022	**Fixed**	0.33		0.14		0.56	A	0.73		0.64	
		NoCCs	0.37		0.51		0.57		1.42		0.88	
		Succession	0.1		0.05		0.26		0.48		0.61	
	Treatment		ns		ns		ns		ns		ns	
	Time		ns		ns		$p<0.001$		ns		ns	
	Treatment x Time		ns		ns		ns		ns		ns	
SU	**2020**	**Fixed**	1.04	A	0.27	ab	0.52	A	0.98	A	-0.71	ab
		NoCCs	-0.54		-1.71	c	-0.15		-0.69		-2.43	c

		Succession	1.10		0.43	ab	0.17		0.24		-0.85	ab
	2021	**Fixed**	-1.55	B	-0.28	ab	-2.22	B	-1.43	B	-1.72	b
		NoCCs	-2.08		-1.39	b	-2.26		-1.29		-1.68	ab
		Succession	-1.68		0.38	ab	-1.70		-1.03		-0.99	ab
	2022	**Fixed**	-0.05	A	1.94	a	0.27	A	1.25	A	0.82	a
		NoCCs	0.17		0.68	ab	-0.33		0.74		0.31	ab
		Succession	0.37		1.40	ab	-0.24		1.06		0.86	a
	Treatment		ns		ns		ns		ns		ns	
	Time		p<0.001		ns		p<0.001		p<0.001		ns	
	Treatment x Time		ns		p<0.01		ns		ns		p<0.001	

Table 2S. Average gene abundances (AOB, AOA, nirkK, nifH, nosZ) in the 20-40 cm soil layer (number of ln gene copies per g soil^{-1}) for each period analysed (cover crop growing season- WI; first 1,5 months after cover crop termination- SP; cash crop growing season- SU), in each year and cover crops treatments. Different letters indicate significant differences (Wald test ANOVA); ns = not significant.

Period	**Year**	**Treatment**	**Genes abundance in the 20-40 cm depth (number of ln gene copies per g soil^{-1})**									
			AOB		**AOA**		**nirK**		**nifH**		**nosZ**	
WI	**2020**	**Fixed**	-0.31	B	-2.17	c	-0.87		-1.99	abc	-0.52	ab
		NoCCs	-0.30		-0.79	abc	0.22		-0.45	abc	0.12	ab
		Succession	-0.50		-2.10	bc	-1.63		-1.84	abc	-0.68	ab
	2021	**Fixed**	3.52	A	0.02	abc	-0.27		-0.45	abc	0.33	ab
		NoCCs	2.59		0.51	ab	1.46		0.61	a	0.83	ab
		Succession	3.81		0.73	a	0.76		0.54	ab	0.91	a
	2022	**Fixed**	-1.54	B	-1.54	abc	-3.24		-2.32	bc	-2.11	b
		NoCCs	-1.29		-1.29	abc	-3.63		-2.73	c	-2.04	ab
		Succession	-1.18		-1.18	abc	-4.22		-2.57	c	-1.58	ab
	Treatment		ns		ns		ns		ns		ns	
	Time		p<0.001		ns		ns		ns		ns	
	Treatment x Time		ns		p<0.001		ns		p<0.001		p<0.001	
SP	**2020**	**Fixed**	1.37	a	3.66	a	2.89	a	3.72	a	2.64	a
		NoCCs	0.04	ab	0.79	abc	0.64	ab	1.47	abc	0.08	ab
		Succession	0.24	ab	2.31	ab	1.71	ab	2.06	ab	0.78	ab
	2021	**Fixed**	-0.42	ab	-0.56	bc	0.01	ab	-0.54	bcd	-0.18	ab
		NoCCs	-0.50	ab	-0.35	bc	-0.14	ab	-0.13	bcd	0.60	ab
		Succession	-1.11	b	-1.25	bc	-1.13	b	-1.53	bcd	-0.75	ab
	2022	**Fixed**	-1.54	b	-3.24	c	-2.32	b	-2.11	d	-2.38	b
		NoCCs	-1.29	b	-3.63	c	-2.73	b	-2.04	cd	-2.43	b
		Succession	-1.18	b	-4.22	c	-2.57	b	-1.58	bcd	-2.38	b
	Treatment		ns		ns		ns		ns		ns	
	Time		ns		ns		ns		ns		ns	
	Treatment x Time		p<0.001		p<0.001		p<0.001		p<0.001		p<0.001	
SU	**2020**	**Fixed**	0.22	ab	-0.63	ab	-0.51		-1.61	cd	-0.31	ab
		NoCCs	-0.95	b	-2.01	b	-1.47		-2.15	d	-1.39	b
		Succession	-0.07	ab	-0.60	ab	-0.09		-1.11	bcd	-0.11	ab
	2021	**Fixed**	0.07	ab	0.02	ab	0.09		-0.15	bcd	0.28	ab
		NoCCs	0.69	a	0.64	ab	-0.03		0.08	acbd	0.41	ab
		Succession	0.98	a	1.36	a	0.75		1.02	ab	1.42	a
	2022	**Fixed**	0.17	ab	1.59	a	0.38		2.40	a	1.27	a
		NoCCs	-0.12	ab	1.49	a	-0.66		0.57	abc	0.35	ab
		Succession	-0.01	ab	0.95	a	-0.66		1.06	ab	0.54	ab
	Treatment		ns		ns		ns		ns		ns	
	Time		ns		ns		ns		ns		ns	
	Treatment x Time		p<0.01		p<0.001		ns		p<0.001		p<0.01	

Table 3S. Average genes' abundance (number of ln gene copies per g soil^{-1}) in the shallower soil layer (0-20 cm depth) of each soil N functional gene (AOA, AOB, nirK, nosZ, nifH) in each year (I, II, III) and cover crops treatment (Fix- triticale for all the three years; Suc- three years succession of rye, clover, mustard). The abundances reported were collected in the WI period (at cover crop termination time). Upper (Upper CL) and lower (Lower CL) 0.95 confidence intervals are reported. Different letters indicate significant differences (p<0.05) between gene types' abundances (Groups).

Year	**GeneType**	**TreatmentType**	**emmean**	**lower.CL**	**upper.CL**	**Significance**
II	AOB	Succession	4.95975561	2.86747444	7.05203679	a
II	AOB	Fixed	3.38502119	1.29274001	5.47730236	ab
II	AOB	NoCCs	2.88490292	0.79262174	4.9771841	abc
I	nosZ	NoCCs	1.22078136	-0.87149982	3.31306254	bcd
II	nifH	Fixed	1.0482918	-1.04398937	3.14057298	bcde
I	nosZ	Succession	0.90807062	-1.18421056	3.00035179	bcde
I	AOA	NoCCs	0.824541	-1.26774018	2.91682217	bcde
I	nosZ	Fixed	0.21129024	-1.88099093	2.30357142	bcdef
II	nirK	NoCCs	0.02477667	-2.06750451	2.11705784	bcdefg
I	AOB	NoCCs	0.0236742	-2.06860698	2.11595538	bcdefg
II	nirK	Fixed	-0.09352505	-2.18580623	1.99875612	bcdefg
II	nirK	Succession	-0.10807661	-2.20035779	1.98420457	bcdefg
I	AOB	Succession	-0.10994561	-2.20222678	1.98233557	bcdefg
I	nifH	NoCCs	-0.15127808	-2.24355925	1.9410031	bcdefg
II	nifH	NoCCs	-0.34473945	-2.43702062	1.74754173	cdefg

I	nirK	NoCCs	-0.40937296	-2.50165413	1.68290822	cdefg
II	nifH	Succession	-0.4530559	-2.54533708	1.63922528	cdefg
I	AOA	Succession	-0.46858878	-2.56086996	1.6236924	cdefg
II	nosZ	Fixed	-0.52749704	-2.61977821	1.56478414	cdefg
I	AOB	Fixed	-0.55309496	-2.64537614	1.53918621	cdefg
I	nirK	Fixed	-0.63580512	-2.72808629	1.45647606	cdefgh
I	nifH	Fixed	-0.80407186	-2.89635304	1.28820932	cdefgh
I	nirK	Succession	-0.82495686	-2.91723803	1.26732432	defgh
III	AOB	Succession	-1.00969346	-3.10197464	1.08258771	defgh
I	nifH	Succession	-1.06854463	-3.16082581	1.02373655	defgh
III	AOB	Fixed	-1.22413509	-3.31641627	0.86814608	defgh
II	AOA	Fixed	-1.31138844	-3.40366961	0.78089274	defgh
I	AOA	Fixed	-1.39464244	-3.48692362	0.69763874	defgh
III	AOB	NoCCs	-1.52336916	-3.61565034	0.56891202	defgh
III	nirK	Succession	-1.65037006	-3.74265124	0.44191111	defgh
II	AOA	Succession	-1.98982785	-4.08210902	0.10245333	defgh
II	nosZ	NoCCs	-2.0950935	-4.18737468	-0.00281232	defgh

III	nirK	Fixed	-2.25964372	-4.3519249	-0.16736254	defgh
III	nosZ	Fixed	-2.34879684	-4.44107802	-0.25651566	defgh
III	nirK	NoCCs	-2.48157834	-4.57385952	-0.38929716	efgh
II	AOA	NoCCs	-2.48352872	-4.5758099	-0.39124754	efgh
III	nifH	Succession	-2.49867453	-4.5909557	-0.40639335	efgh
III	nosZ	Succession	-2.56174983	-4.654031	-0.46946865	efgh
III	nifH	Fixed	-2.63234116	-4.72462234	-0.54005999	efgh
III	nosZ	NoCCs	-2.91603626	-5.00831744	-0.82375509	fgh
III	nifH	NoCCs	-3.21061086	-5.30289204	-1.11832968	fgh
III	AOA	NoCCs	-3.30947868	-5.40175986	-1.21719751	fgh
III	AOA	Fixed	-3.33863224	-5.43091342	-1.24635107	fgh
III	AOA	Succession	-3.59494374	-5.68722491	-1.50266256	gh
II	nosZ	Succession	-4.26194618	-6.35422736	-2.169665	h
I	nirK	NoCCs	-0.40937296	-2.50165413	1.68290822	cdefg

Table 4S. Average genes' abundance (number of ln gene copies per g soil^{-1}) in the shallower soil layer (20-40 cm depth) of each soil N functional gene (AOA, AOB, nirK, nosZ, nifH) in each year (I, II, III) and cover crops treatment (Fix- triticale for all the three years; Suc- three years succession of rye, clover, mustard). The abundances reported were collected in the SU period (at cash crop harvest time). Upper (Upper CL) and lower (Lower CL) 0.95 confidence intervals are reported. Different letters indicate significant differences (p<0.05) between gene types' abundances (Groups).

Year	**GeneType**	**TreatmentType**	**emmean**	**lower.CL**	**upper.CL**	**Significance**
III	AOB	Fixed	1.94299838	0.19983499	3.68616178	a
III	AOB	Succession	1.39561311	-0.34755029	3.1387765	ab
III	nirK	Fixed	1.25159479	-0.4915686	2.99475819	abc
I	AOA	Succession	1.09892306	-0.64424033	2.84208645	abc
III	nirK	Succession	1.05658511	-0.68657828	2.7997485	abc
I	AOA	Fixed	1.03653736	-0.70662604	2.77970075	abc
I	nirK	Fixed	0.97933107	-0.76383232	2.72249446	abcd
III	nosZ	Succession	0.85586307	-0.88730032	2.59902647	abcd
III	nosZ	Fixed	0.81992093	-0.92324246	2.56308433	abcde
III	nirK	NoCCs	0.74142386	-1.00173953	2.48458726	abcdef
III	AOB	NoCCs	0.6845662	-1.05859719	2.42772959	abcdef
I	nifH	Fixed	0.5172913	-1.22587209	2.26045469	abcdefg
I	AOB	Succession	0.43212042	-1.31104297	2.17528381	abcdefg
II	AOB	Succession	0.37553029	-1.3676331	2.11869369	abcdefg
III	AOA	Succession	0.37374147	-1.36942192	2.11690486	abcdefg

III	nosZ	NoCCs	0.30904574	-1.43411765	2.05220913	abcdefg
III	nifH	Fixed	0.26708272	-1.47608067	2.01024612	abcdefg
I	AOB	Fixed	0.26561814	-1.47754526	2.00878153	abcdefg
I	nirK	Succession	0.23849964	-1.50466375	1.98166303	abcdefg
I	nifH	Succession	0.17321841	-1.56994498	1.91638181	abcdefg
III	AOA	NoCCs	0.1730744	-1.570089	1.91623779	abcdefg
III	AOA	Fixed	-0.05020846	-1.79337185	1.69295493	abcdefg
I	nifH	NoCCs	-0.15163979	-1.89480318	1.59152361	abcdefg
III	nifH	Succession	-0.24469382	-1.98785722	1.49846957	abcdefg
II	AOB	Fixed	-0.27536983	-2.01853322	1.46779357	abcdefg
III	nifH	NoCCs	-0.33367904	-2.07684243	1.40948436	abcdefg
I	AOA	NoCCs	-0.53675155	-2.27991495	1.20641184	abcdefg
I	nirK	NoCCs	-0.69464906	-2.43781246	1.04851433	abcdefg
I	nosZ	Fixed	-0.71067152	-2.45383492	1.03249187	abcdefg
I	nosZ	Succession	-0.84894103	-2.59210442	0.89422236	abcdefg
II	nosZ	Succession	-0.99104796	-2.73421136	0.75211543	abcdefg
II	nirK	Succession	-1.03238551	-2.7755489	0.71077788	abcdefg

II	nirK	NoCCs	-1.29326823	-3.03643162	0.44989516	bcdefg
II	AOB	NoCCs	-1.3930401	-3.13620349	0.3501233	bcdefg
II	nirK	Fixed	-1.42762387	-3.17078726	0.31553952	bcdefg
II	AOA	Fixed	-1.54987682	-3.29304022	0.19328657	bcdefg
II	nosZ	NoCCs	-1.68062003	-3.42378343	0.06254336	cdefg
II	AOA	Succession	-1.68380081	-3.4269642	0.05936259	cdefg
II	nifH	Succession	-1.70362378	-3.44678717	0.03953962	cdefg
I	AOB	NoCCs	-1.70574503	-3.44890842	0.03741837	cdefg
II	nosZ	Fixed	-1.71821266	-3.46137605	0.02495074	cdefg
II	AOA	NoCCs	-2.07699802	-3.82016141	-0.33383463	defg
II	nifH	Fixed	-2.22379083	-3.96695422	-0.48062743	efg
II	nifH	NoCCs	-2.26260266	-4.00576606	-0.51943927	fg
I	nosZ	NoCCs	-2.43003347	-4.17319686	-0.68687007	g

Table 5S. Soil water content average values (emmeans) at 0-20 cm depth during the Winter Period (WI) of the II year of experimentation (2020-2021). Standard Error (SE); upper and lower confidence interval (CL) (at 0.95 confidence interval). Different letters indicate significant differences ($p<0.05$) between the levels of the source of variation.

Date	**emmean**	**lower.CL**	**upper.CL**	**Significance**
2020-10-07	3.80006817	-3.951378334	11.55151467	f
2020-10-13	8.55630833	0.804861833	16.30775483	bcdef
2020-10-20	10.4422776	2.690831083	18.19372408	bcdef
2020-10-28	9.69317858	1.941732083	17.44462508	bcdef
2020-11-05	7.65780842	-0.093638084	15.40925492	def
2020-11-12	6.51777617	-1.233670334	14.26922267	ef
2020-11-18	9.17772367	1.426277166	16.92917017	bcdef
2020-11-26	8.16625683	0.414810333	15.91770333	cdef
2020-12-04	15.6394084	7.887961916	23.39085492	abcdef
2020-12-11	20.6221908	12.87074425	28.37363725	abcd
2020-12-17	18.3869408	10.63549425	26.13838725	abcde
2020-12-21	17.5175158	9.766069249	25.26896225	abcde
2021-01-07	21.5741074	13.82266092	29.32555392	abc
2021-01-16	17.1546074	9.403160916	24.90605392	abcdef
2021-01-20	16.8107741	9.059327583	24.56222058	abcdef
2021-01-29	19.7504408	11.99899425	27.50188725	abcde
2021-02-03	21.7614408	14.00999425	29.51288725	ab
2021-02-11	24.0452741	16.29382758	31.79672058	a
2021-02-17	18.4772741	10.72582758	26.22872058	abcde
2021-02-25	16.7645241	9.013077583	24.51597058	abcdef
2021-03-05	13.7783574	6.026910916	21.52980392	abcdef
2021-03-10	13.0749408	5.323494249	20.82638725	abcdef
2021-03-17	10.9458574	3.194410916	18.69730392	abcdef
2021-03-25	8.17802408	0.426577583	15.92947058	cdef

Table 6S. Soil water content average values (emmeans) at 20-40 cm depth during the Winter Period (WI) of the II year of experimentation (2020-2021). Standard Error (SE); upper and lower confidence interval (CI) (at 0.95 confidence interval). Different letters indicate significant differences ($p<0.05$) between the levels of the source of variation.

Date	**emmean**	**SE**	**lower.CL**	**upper.CL**	**Significance**
2020-10-07	-4.059984889	4.911006996	-19.3247299	11.20476012	e
2020-10-13	-3.516497444	4.911006996	-18.78124245	11.74824756	e
2020-10-20	2.416554111	4.911006996	-12.8481909	17.68129912	bcde
2020-10-28	2.626752667	4.911006996	-12.63799234	17.89149767	bcde
2020-11-05	1.447595556	4.911006996	-13.81714945	16.71234056	cde
2020-11-12	-0.076399778	4.911006996	-15.34114479	15.18834523	de
2020-11-18	0.328418444	4.911006996	-14.93632656	15.59316345	cde
2020-11-26	0.903471667	4.911006996	-14.36127334	16.16821667	cde
2020-12-04	6.278610333	4.911006996	-8.986134674	21.54335534	abcde
2020-12-11	24.357091	4.911006996	9.092345992	39.62183601	abcd
2020-12-17	20.27342433	4.911006996	5.008679326	35.53816934	abcde
2020-12-21	18.969191	4.911006996	3.704445992	34.23393601	abcde
2021-01-07	26.68786878	4.911006996	11.42312377	41.95261379	abc
2021-01-16	20.543091	4.911006996	5.278345992	35.80783601	abcde
2021-01-20	19.92053544	4.911006996	4.655790437	35.18528045	abcde
2021-01-29	25.10853544	4.911006996	9.843790437	40.37328045	abcd
2021-02-03	28.382091	4.911006996	13.11734599	43.64683601	ab
2021-02-11	31.48564656	4.911006996	16.22090155	46.75039156	a
2021-02-17	23.65675767	4.911006996	8.392012659	38.92150267	abcd
2021-02-25	21.78986878	4.911006996	6.52512377	37.05461379	abcde
2021-03-05	18.45442433	4.911006996	3.189679326	33.71916934	abcde
2021-03-10	16.91864656	4.911006996	1.653901548	32.18339156	abcde

2021-03-17	14.26953544	4.911006996	-0.995209563	29.53428045	abcde
2021-03-25	14.95697989	4.911006996	-4.307765119	26.2217249	abcde

Table 7S. Soil water content average values (emmeans) at 0-20 cm depth during the Winter Period (WI) of the III year of experimentation (2021-2022). Standard Error (SE); upper and lower confidence interval (CI) (at 0.95 confidence interval). Different letters indicate significant differences ($p<0.05$) between the levels of the source of variation.

Date	emmean	SE	lower.CL	upper.CL	Significance
2021-10-02	2.01	2.767884074	-6.627717941	10.64956583	bcd
2021-10-07	-3.01	2.767884074	-11.64630286	5.630980914	cd
2021-10-15	-4.92	2.767884074	-13.55649547	3.720788302	d
2021-10-21	-3.47	2.767884074	-12.10409027	5.173193497	cd
2021-10-27	13.34	2.767884074	4.696696614	21.97398039	ab
2021-11-04	8.46	2.767884074	-0.175414386	17.10186939	abcd
2021-11-12	11.01	2.767884074	2.372125753	19.64940952	abc
2021-11-18	9.04	2.767884074	0.404627281	17.68191105	abcd
2021-11-25	14.82	2.767884074	6.185164448	23.46244822	ab
2021-12-07	17.40	2.767884074	8.763789309	26.04107308	a
2021-12-15	15.31	2.625845392	7.112521808	23.50319236	ab
2021-12-22	13.73	2.625845392	5.535976308	21.92664686	ab
2021-12-29	15.20	2.625845392	7.008475892	23.39914644	ab
2022-01-05	13.68	2.625845392	5.489563058	21.88023361	ab
2022-01-12	15.53	2.625845392	7.331588475	23.72225902	ab
2022-01-20	14.20	2.625845392	6.004550142	22.39522069	ab
2022-01-26	13.56	2.625845392	5.368286725	21.75895727	ab
2022-02-02	12.13	2.625845392	3.936622058	20.32729261	ab
2022-02-10	10.62	2.625845392	2.421314058	18.81198461	abc
2022-02-16	12.97	2.625845392	4.777838475	21.16850902	ab
2022-02-24	10.44	2.625845392	2.243715225	18.63438577	abc
2022-03-03	10.86	2.625845392	1.290067975	17.68073852	abcd
2022-03-10	10.59	2.625845392	-1.753846358	14.63682419	abcd
2022-03-17	10.32	2.625845392	-3.165592608	13.22507794	abcd
2022-03-23	10.04	2.625845392	-5.303190108	11.08748044	abcd

Table 8S. Soil water content average values (emmeans) at 20-40 cm depth during the Winter Period (WI) of the III year of experimentation (2021-2022). Standard Error (SE); upper and lower confidence interval (CI) (at 0.95 confidence interval). Different letters indicate significant differences (p<0.05) between the levels of the source of variation.

Date	**emmean**	**SE**	**lower.CL**	**upper.CL**	**Significance**
2021-10-02	-23.26067252	5.5653	-40.6303	-5.8911	c
2021-10-07	-24.03762074	5.5653	-41.4072	-6.6680	c
2021-10-15	-24.0403353	5.5653	-41.4099	-6.6707	c
2021-10-21	-19.62211185	5.5653	-36.9917	-2.2525	bc
2021-10-27	1.056833889	5.5653	-16.3128	18.4264	abc
2021-11-04	-2.592404815	5.5653	-19.9620	14.7772	abc
2021-11-12	-0.959383481	5.5653	-18.3290	16.4102	abc
2021-11-18	-0.985546	5.5653	-18.3551	16.3840	abc
2021-11-25	7.547040852	5.5653	-9.8226	24.9166	ab
2021-12-07	12.28616889	5.5653	-5.0834	29.6558	a
2021-12-15	9.232191667	5.2797	-7.2461	25.7104	ab
2021-12-22	7.185518556	5.2797	-9.2927	23.6638	ab
2021-12-29	8.125275444	5.2797	-8.3530	24.6035	ab
2022-01-05	7.719740667	5.2797	-8.7585	24.1980	ab
2022-01-12	15.20518222	5.2797	-4.8731	28.0834	a
2022-01-20	9.244710778	5.2797	-7.2335	25.7230	ab
2022-01-26	7.961157	5.2797	-8.5171	24.4394	ab
2022-02-02	6.252626	5.2797	-10.2256	22.7309	ab
2022-02-10	4.518277333	5.2797	-11.9600	20.9965	abc
2022-02-16	4.686535	5.2797	-11.7917	21.1648	abc
2022-02-24	3.716183556	5.2797	-12.7621	20.1944	abc
2022-03-03	2.544458667	5.2797	-13.9338	19.0227	abc
2022-03-10	-0.748404556	5.2797	-17.2266	15.7298	abc
2022-03-17	-2.761619444	5.2797	-19.2399	13.7166	abc
2022-03-23	-2.726401889	5.2797	-21.2046	11.7518	abc

Table 9S. Soil water content average values (emmeans) at 0-20 cm depth during the period spanning from the cover crops termination until the subsequent cash crop harvest (2020) Standard Error (SE); upper and lower confidence interval (CI) (at 0.95 confidence interval). Different letters indicate significant differences (p<0.05) between the levels of the source of variation.

Date	**emmean**	**SE**	**lower.CL**	**upper.CL**	**Significance**
2020-06-01	-1.098824833	1.974625252	-6.765902754	4.568253087	d
2020-06-05	14.77685233	1.974625252	9.109774413	20.44393025	ab
2020-06-12	16.96291817	1.974625252	11.29584025	22.62999609	a
2020-06-22	12.63051967	1.974625252	6.963441746	18.29759759	abc
2020-06-29	4.68717225	1.974625252	-0.97990567	10.35425017	cd
2020-07-03	3.112201583	1.974625252	-2.554876337	8.779279504	d
2020-07-08	7.552161417	1.974625252	1.885083496	13.21923934	bcd
2020-07-14	1.485772833	1.974625252	-4.181305087	7.152850754	d
2020-08-05	19.15560425	1.974625252	13.48852633	24.82268217	a
2020-08-26	16.403318	1.974625252	10.73624008	22.07039592	ab
2020-09-28	-0.84	1.974625252	-14.7	15.7	d

Table 10S. Soil water content average values (emmeans) at 20-40 cm depth during the period spanning from the cover crops termination until the subsequent cash crop harvest (2020) Standard Error (SE); upper and lower confidence interval (CI) (at 0.95 confidence interval). Different letters indicate significant differences (p<0.05) between the levels of the source of variation.

Date	**emmean**	**SE**	**lower.CL**	**upper.CL**	**Significance**
2020-06-01	-2.5031082	3.980205403	-13.92610311	8.919886665	c
2020-06-05	7.96031678	3.980205403	-3.462678109	19.38331166	bc
2020-06-12	28.0074506	3.980205403	16.58445567	39.43044544	a
2020-06-22	20.8034516	3.980205403	9.380456669	32.22644644	ab
2020-06-29	7.00185789	3.980205403	-4.421136998	18.42485278	bc
2020-07-03	5.13035233	3.980205403	-6.292642554	16.55334722	bc
2020-07-08	8.31787922	3.980205403	-3.105115665	19.74087411	bc
2020-07-14	-1.3030549	3.980205403	-12.72604978	10.11994	c
2020-08-05	12.1960668	3.980205403	0.773071891	23.61906166	abc
2020-08-26	12.5868108	3.980205403	1.163815891	24.00980566	abc
2020-09-28	-9.6593140	3.980205403	-17.72604978	-1.75630100	d

Table 11S. Soil water content average values (emmeans) at 0-20 cm depth during the period spanning from the cover crops termination until the subsequent cash crop harvest (2021) Standard Error (SE); upper and lower confidence interval (CI) (at 0.95 confidence interval). Different letters indicate significant differences (p<0.05) between the levels of the source of variation.

Date	**emmean**	**SE**	**lower.CL**	**upper.CL**	**Significance**
2021-04-08	6.284197083	2.263254065	-0.573917045	13.14231121	cde
2021-04-14	24.07104758	2.263254065	17.21293346	30.92916171	a
2021-04-20	14.81696458	2.263254065	7.958850455	21.67507871	abc
2021-04-26	21.64114233	2.263254065	14.78302821	28.49925646	ab
2021-04-29	10.838619	2.263254065	3.980504872	17.69673313	bcde
2021-05-05	13.82008075	2.263254065	6.961966622	20.67819488	abcd
2021-06-10	7.558780083	2.263254065	0.700665955	14.41689421	cde
2021-06-15	3.380688583	2.263254065	-3.477425545	10.23880271	cdef
2021-06-23	0.386199167	2.263254065	-6.471914961	7.244313295	efgh
2021-06-30	2.041614167	2.263254065	-4.816499961	8.899728295	efg
2021-07-09	11.15427975	2.263254065	4.296165622	18.01239388	bcde
2021-07-15	2.169787833	2.263254065	-4.688326295	9.027901961	defg
2021-07-22	-0.277149333	2.263254065	-7.135263461	6.580964795	efgh
2021-07-28	-0.570597917	2.263254065	-7.428712045	6.287516211	efgh
2021-08-25	-7.727191167	2.263254065	-14.58530529	-0.869077039	fghi
2021-09-03	-11.23981275	2.263254065	-18.09792688	-4.381698622	hi
2021-09-10	-13.26963925	2.263254065	-20.12775338	-6.411525122	i
2021-09-18	-9.310167917	2.263254065	-16.16828204	-2.452053789	ghi

Table 12S. Soil water content average values (emmeans) at 20-40 cm depth during the period spanning from the cover crops termination until the subsequent cash crop harvest (2021) Standard Error (SE); upper and lower confidence interval (CI) (at 0.95 confidence interval). Different letters indicate significant differences (p<0.05) between the levels of the source of variation.

Date	emmean	SE	lower.CL	upper.CL	Significance
2021-04-08	7.43302989	4.131517613	-5.086297813	19.95235759	bcdef
2021-04-14	32.5359379	4.131517613	20.01661019	45.05526559	a
2021-04-20	21.7567037	4.131517613	9.237375965	34.27603137	ab
2021-04-26	33.6750681	4.131517613	21.15574041	46.19439581	a
2021-04-29	16.1434697	4.131517613	3.624141965	28.66279737	abcd
2021-05-05	19.200267	4.131517613	6.680939298	31.7195947	abc
2021-06-10	14.0379411	4.131517613	1.518613409	26.55726881	abcde
2021-06-15	7.737219	4.131517613	-4.782108702	20.2565467	bcdef
2021-06-23	1.32062389	4.131517613	-11.19870381	13.83995159	bcdef
2021-06-30	-0.96473156	4.131517613	-13.48405926	11.55459615	cdef
2021-07-09	8.83431856	4.131517613	-3.685009146	21.35364626	bcdef
2021-07-15	-2.463871	4.131517613	-14.9831987	10.0554567	def
2021-07-22	-6.08224811	4.131517613	-18.60157581	6.437079591	ef
2021-07-28	-11.1418561	4.131517613	-23.66118381	1.377471591	fg
2021-08-25	-27.8292439	4.131517613	-40.34857159	-15.30991619	g
2021-09-03	-30.1154606	4.131517613	-42.63478826	-17.59613285	g
2021-09-10	-32.0202001	4.131517613	-44.53952781	-19.50087241	g
2021-09-18	-30.9222329	4.131517613	-43.44156059	-18.40290519	g

Table 13S. Soil water content average values (emmeans) at 0-20 cm depth during the period spanning from the cover crops termination until the subsequent cash crop harvest (2022) Standard Error (SE); upper and lower confidence interval (CI) (at 0.95 confidence interval). Different letters indicate significant differences ($p<0.05$) between the levels of the source of variation.

Date	emmean	SE	lower.CL	upper.CL	Significance
2022-04-05	4.14241575	2.618677287	-3.902375245	12.18720674	ab
2022-04-14	-1.16223583	2.618677287	-9.207026828	6.882555161	abc

2022-04-20	-3.59398542	2.618677287	-11.63877641	4.450805578	abc
2022-04-28	7.26185492	2.618677287	-0.782936078	15.30664591	a
2022-05-04	-0.95755817	2.618677287	-9.002349161	7.087232828	abc
2022-05-12	2.119816	2.618677287	-5.924974995	10.16460699	ab
2022-05-19	-1.4335155	2.618677287	-9.478306495	6.611275495	abc
2022-05-25	-1.49728692	2.618677287	-9.542077911	6.547504078	abc
2022-05-31	-3.07951217	2.618677287	-11.12430316	4.965278828	abc
2022-06-09	3.80155525	2.618677287	-4.243235745	11.84634624	ab
2022-06-15	0.95109675	2.618677287	-7.093694245	8.995887745	abc
2022-06-22	-3.19756492	2.618677287	-11.24235591	4.847226078	abc
2022-06-28	-4.023558	2.618677287	-12.06834899	4.021232995	abc
2022-07-05	-5.09160392	2.618677287	-13.13639491	2.953187078	abc
2022-07-14	2.15414083	2.618677287	-5.890650161	10.19893183	ab
2022-07-21	-2.71768617	2.618677287	-10.76247716	5.327104828	abc
2022-07-28	-9.60488667	2.618677287	-17.64967766	-1.560095672	bc
2022-08-04	-12.1497791	2.618677287	-20.19457008	-4.104988089	c
2022-09-07	-13.264863	2.618677287	-21.65429001	-3.65172301	c

Table 14S. Soil water content average values (emmeans) at 20-40 cm depth during the period spanning from the cover crops termination until the subsequent cash crop harvest (2022) Standard Error (SE); upper and lower confidence interval (CI) (at 0.95 confidence interval). Different letters indicate significant differences ($p<0.05$) between the levels of the source of variation.

Date	**emmean**	**SE**	**lower.CL**	**upper.CL**	**Significance**
2022-04-05	-4.895174222	4.71290486	-19.37360376	9.583255316	a
2022-04-28	-7.846275333	4.71290486	-22.32470487	6.632154205	ab
2022-05-12	-8.344015556	4.71290486	-22.82244509	6.134413983	ab
2022-06-09	-8.415280444	4.71290486	-22.89370998	6.063149094	ab

2022-06-15	-9.070917778	4.71290486	-23.54934732	5.40751176	ab
2022-04-14	-10.28875778	4.71290486	-24.76718732	4.18967176	ab
2022-05-25	-10.48043478	4.71290486	-24.95886432	3.99799476	ab
2022-05-04	-10.88368789	4.71290486	-25.36211743	3.594741649	ab
2022-05-19	-11.36630022	4.71290486	-25.84472976	3.112129316	ab
2022-06-22	-11.72928789	4.71290486	-26.20771743	2.749141649	ab
2022-05-31	-12.18164011	4.71290486	-26.66006965	2.296789427	ab
2022-04-20	-13.46412089	4.71290486	-27.94255043	1.014308649	ab
2022-06-28	-13.53185444	4.71290486	-28.01028398	0.946575094	ab
2022-07-14	-13.81566611	4.71290486	-28.29409565	0.662763427	ab
2022-07-21	-15.84216256	4.71290486	-30.32059209	-1.363733017	ab
2022-07-05	-17.75153411	4.71290486	-32.22996365	-3.273104573	ab
2022-08-24	-19.81097256	4.71290486	-34.28940209	-5.332543017	ab
2022-08-31	-23.29864367	4.71290486	-37.7770732	-8.820214128	b
2022-09-07	-25.91	4.71290486	-40.3	-2.41	b

Chapter 4

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RESEARCH ARTICLE

Agronomic management strategies to increase soil organic carbon in the short-term: evidence from on-farm experimentation in the Veneto region

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Abstract

Background and aims Organic fertilizers and cover crops (CC) are considered crucial strategies to pursue the objective of increasing soil organic carbon (SOC). The present research focuses on an 'on farm experimentation' to assess the combined effects of organic fertilization with different biomasses, CC and irrigation on SOC stock.

Methods A 4-year on-farm experimentation was co-developed with local farmers and a land reclamation authority in north-eastern Italy on a biennial maize-soybean rotation. We examined the effects of two organic fertilizers (compost or digestate), three CC treatments (a fixed cover crop species – *x triticosecale*; a succession of cover crop species – *Sinapis alba* and *Lolium multiflorum*; no CC) under rainfed and irrigated conditions on the SOC content and stock, and crops yields.

Results All these integrated practices – except when digestate was applied in the field in the absence of a CC under rainfed conditions – determined a significant increase of the SOC stock after 4 years, matching the goals set by the '*4 per mille*' initiative. The highest SOC increase was observed under irrigated management and compost fertilization, regardless of the presence or absence of a CC (range: 9.3–10.3 Mg ha^{-1} in the first 0–40 cm of soil). Soybean grain yields were comparable with those obtained in farms of the same rural district under business as usual, but maize grain yields were lower.

Conclusion SOC accumulation is achievable in the short term with abundant applications of organic biomass, but the strategy might lead to economic loss such as lower maize productivity.

Keywords Digestate · Compost · Irrigation · Cover crops · Maize-soybean rotation · Organic matter

Introduction

Promoting soil organic carbon (SOC) storage, considered as a proxy for soil organic matter (SOM)

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accumulation and soil health, is crucial in the path towards a more resilient agriculture able to mitigate climate change (Lal 2004). At COP21 (Paris 2015), the '4 per mille Soils for Food Security and Climate' was launched to increase global SOM by 4 per 1000 per year to compensate for anthropogenic greenhouse gas emissions (Rhodes 2016; Rumpel et al. 2020; https://4p1000.org/?lang=en). In the same perspective, the EIP-AGRI Focus Group 'Soil Organic Matter in Mediterranean regions' brought together scientists with different expertise to formulate valuable and feasible solutions to improve SOM in the Mediterranean area in order to overcome the excessively widespread condition of soils with an organic carbon (OC) content lower than 1% in southern Europe (Zdruli et al. 2004). The SOC stock was proposed as an indicator to monitor land and soil degradation. Supplementing the soil by returning the cash crop residues and cover crop (CC) biomass, together with external OC sources (digestate, manure, compost) is considered as a complementary strategy with a potential to increase SOC in many agroecosystems (Costantini et al. 2020) and has been listed among the best practices by the EIP-AGRI working group on SOM. The increase of the SOC stock can contribute to Agenda 2030 for Sustainable Development (Lal 2016; Soussana et al. 2019) to reach Target 2.4 '*By 2030, ensure sustainable food production systems and implement resilient agricultural practices that increase productivity and production, that help maintain ecosystems, that strengthen capacity for adaptation to climate change, extreme weather, drought, flooding and other disasters and that progressively improve land and soil quality*' and Target 15.3 '*By 2030, combat desertification, restore degraded land and soil, including land affected by desertification, drought and floods, and strive to achieve a land degradation-neutral world*'.

The spotlight on SOM is due to its benefits in agroecosystems, related to the three dimensions of soil quality and fertility. From a chemical perspective, SOM significantly contributes to the nutrient storage and supply capacity of soils, soil pH buffering capacity, and retention of pollutants or toxic elements (Bartóg et al. 2020); from a physical one, it contributes to the soil structure and thereby to ultimately control soil erosion, water infiltration and the water-holding capacity (Lal 2020); biologically speaking, it is a primary source of carbon (C)/energy for soil microorganisms and for the whole soil biota, which are key players in soil function, while soils are one of the largest reservoirs of biodiversity (Martínez-García et al. 2018).

In agroecosystems, SOC accumulation is dependent on the balance of biomass C inputs and C losses through mineralization, leaching, and erosion (Liu et al. 2006). Therefore, agricultural management practices influence SOC accumulation greatly (Basso 2022) for example soil tillage (Mazzoncini et al. 2016), management and fertilizer choices (Bhogal et al. 2018), crop residue management (Turmel et al. 2015) and crop rotations (Dal Ferro et al. 2020), including the integration of CCs between consecutive cash crops (Thapa et al. 2022). Despite great interest in the topic of increasing the SOC stock, there is still great uncertainty about the efficacy of some practices because of highly variable effects among and within practices (Chenu et al. 2019) due to site specific climatic and soil conditions, as well as the accuracy of scaling up results from the microcosm to plot, field or even basin scale (Dignac et al. 2017). For example, some works report greater efficacy of compost than digestate for a more rapid increase of SOC (Bhogal et al. 2018), as well as different contributions of CC species to the SOC stock (Higashi et al. 2014) and the irrigation management (Emde et al. 2021). Moreover, in recent years, low rainfall and high weather variability have accelerated SOM losses (Pérez-Guzmán et al. 2020) and increased the pressure on farmers when it comes to facing the challenge of increasing the SOC stock under climate change scenarios. As highlighted by the EIP-AGRI Focus Group 'Soil Organic Matter in Mediterranean regions', it is urgent to increase SOC in degraded soils (SOC < 1%) in the short term, especially in the upper soil layer. However, the SOC stock increase is highly dependent on the time span and pedoclimatic conditions (Tadiello et al. 2023) and not always observed in the short term in large-scale studies also adopting conservation agricultural practices (Camarotto et al. 2020). For this reason, it is necessary to develop integrated agronomic strategies able to increase the SOC stock within a short time that could be easily adopted by farmers, to increase their efficacy and scalability. Considering all the above reported aspects, an on-farm experimentation was designed.

On-farm experimentations are joint explorations in which researchers and others engage closely with farming realities to align with the ways farmers learn (Lacoste et al. 2022). In the present case, the on-farm experiment was co-designed with local stakeholders (farmers and a land reclamation authority) to assess the combined effects of organic fertilization with different biomass sources (compost and digestate), cover crops and irrigation on the SOC stock in the short term.

Materials and methods

Site description

The experimental site (Fig. 1) was located in the demo farm "Podere Fiorentina" of the local Land Reclamation Authority (Consorzio di Bonifica Veneto Orientale – CBVO), in San Donà di Piave (45°38′13.10" N, 12° 35′ 55.00″E, 1 m a.s.l.), north-eastern Italy. The experimental area covered a surface 6.5 ha and was divided in two section – i) irrigated and ii) rainfed. The irrigated sector extended over 4.5 ha, was rectangular shaped and drained with subsurface pipes; the rainfed sector was triangular shaped and composed of four fields drained by a surface system based on ditches. The area falls within the Cfa class of the Köppen classification, with rainfall mainly concentrated in the months of spring and autumn, and frequent thunderstorms during hot-humid summers. Climate data from 1992 to 2022 collected from the Veneto region agency for environmental protection (ARPAV) showed an average annual rainfall of 966 mm and average temperature of 13.7 °C (average maximum and minimum temperatures of 19.1 and 8.9 °C, respectively). The month with the lowest average minimum temperature was January (−0.4 °C), while the month with the highest average maximum temperature was July (30.0 °C). The main physical and chemical soil characteristics for the topsoil layer (0–40 cm) of the experimental site at the start of the experiment are presented in Table 1. It is worth noting that the rainfed area was characterized by higher values of organic carbon, total Kjeldahl nitrogen (TKN) and phosphorus (P). The soil hydrological properties were similar across the experimental area, with a mean bulk density (BD) of 1.25 g cm^{-3}, a mean field capacity of 27.7% (v/v), and a mean wilting point of 8.5% (v/v) in the first 0–40 cm of soil.

Experimental layout and crop management

The experimental layout included 10 plots (0.3 to 0.9 ha). It was co-designed with the local Land Reclamation authority, namely Consorzio di Bonifica Veneto Orientale, and companies working on irrigation (Netafim), seed production (Seminart SRL and Corteva Agriscience ™) and organic matrices production (Bioman SPA) (Fig. 2); the second step consisted in presenting and discussing the concept idea with professionals and farmers during dedicated meetings.

The following variables were studied in the first two years of the experiment: two types of organic

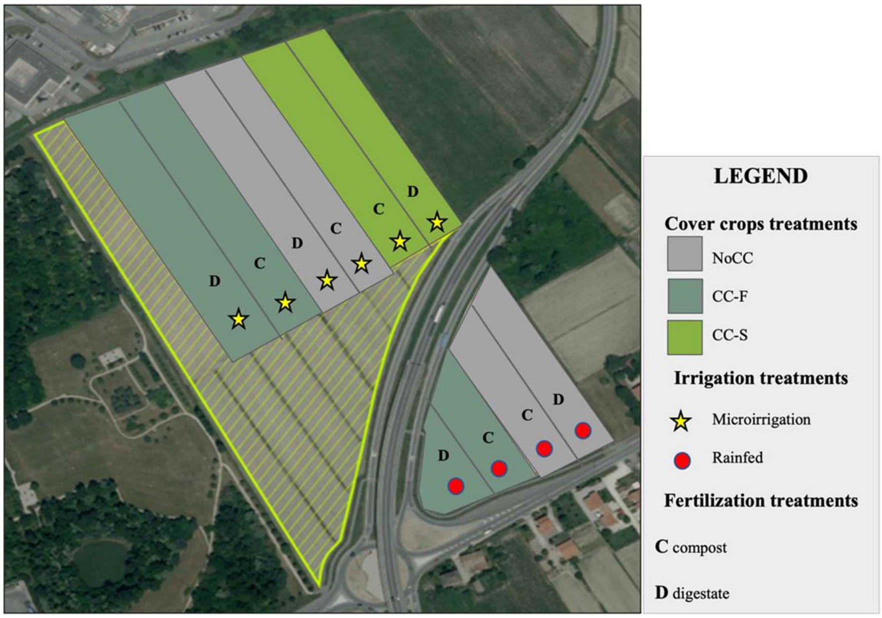

Fig. 1 Map of the "on farm experimentation"

Table 1 Average physico-chemical characteristics of the 0–40 cm soil profile at the beginning of the experimental period (average ± SE)

Soil variables	Field area		Method
	Irrigated	Rainfed	
Sand (%)	51.1 ± 1.0	47.6 ± 1.7	Standard sieve-pipette method (ISO 11277, 2009)
Silt (%)	25.6 ± 0.7	26.2 ± 0.6	
Clay (%)	23.3 ± 0.4	26.2 ± 1.1	
pH	8.14 ± 0.01	8.08 ± 0.01	Dual meter pH/conductivity (soil/water solution with ratio of 1:2.5)
EC (mS cm^{-1})	105.0 ± 0.8	109.5 ± 2.2	
Organic carbon (%)	0.85 ± 0.01	1.13 ± 0.02	CNS elemental analyzer
Inorganic carbon (%)	6.58 ± 0.03	6.34 ± 0.06	
Total Kjeldahl nitrogen (mg kg^{-1})	863.5 ± 15.0	1206.2 ± 25.2	Kjeldahl method
Total phosphorus (mg kg^{-1})	574.3 ± 8.7	706.0 ± 10.4	ICP-OES (Yang et al. 2018)
PO_4^{3-} (mg kg^{-1})	11.4 ± 0.5	18.5 ± 1.2	Ion chromatography system after soil water extraction
NO_3^- (mg kg^{-1})	1.43 ± 0.02	1.15 ± 0.02	

Fig. 2 Discussion about on farm experimentation among researchers and representatives of companies and stakeholders (Land Reclamation Authority, Netafim, Seminart SRL, Corteva Agricscience ™, Bioman SPA) involved in the participatory process

fertilizer: compost from pruning waste (C) vs. digestate from anaerobic digestion of manure (D); ii) two irrigation regimes: drip irrigation (I) vs. rainfed (R). The CC variable was added in the 3rd and 4th years, as follows: a fixed treatment (CC-F) with a species belonging to the Poaceae family (X *triticosecale*) kept constant throughout the experiment as well as a control without a CC (NoCC) in both irrigated and rainfed sections; a 2-year succession (CC-S) of 2 CC species belonging to the Brassicaceae and Poaceae families (*Sinapis alba* L.; *Lolium multiflorum* Lam.) (only in the irrigated section). The distribution of the experimental variables is listed in Table 2.

A grain maize-soybean cash crop succession was adopted throughout the 4 years of the experiment, with the following details: i) grain maize (Pioneer 937 - FAO 700) sown on June 5th 2019 and harvested on October 25th 2019; ii) soybean (var. P21T45) sown on May 9th 2020 and harvested on October 19th 2020; iii) grain maize (Pioneer 937 - FAO 700) sown on April 21st 2021 and harvested on September 23rd 2021; iv) soybean (Pioneer P 18A02) sown on May 11th 2022 and harvested on October 7th 2022. During April of each year, the seedbed preparation for all the cash crops was carried out in spring as follows: organic matrix application using a manure spreader, plowing (about 20 cm depth), subsoil tillage followed by rolling harrowing. During the 3rd and 4th years, CC mechanical termination was performed before organic matter distribution.

The quantity of organic matrices applied was calculated considering their N content and the maximum N application allowed by the regional law (DGR 25 of 2 March 2018). Thus, on yearly average, 9.8 Mg ha^{-1} and 19.0 Mg ha^{-1} of digestate and compost dry matter, respectively, were applied. The costs related to the different fertilization strategies (digestate, compost) was calculated considering cost of purchase, transport and distribution and were compared to those of mineral fertilization. The cost estimation for each fertilization strategy has been set *per* N unit.

During the cash crop cycle, weed control was performed chemically, using post-emergence

Table 2 Description of the integrated practices tested in the on-farm experimentation from 2019 to 2022

Treatment code	Agronomic practices			
	Organic fertilization 2019–2022	Irrigation 2019–2022	Cover crop 2020/2021	Cover crop 2021/2022
CC-F:D:I	Digestate	Microirrigation	Triticale	Triticale
CC-F:C:I	Compost	Microirrigation	Triticale	Triticale
NoCC:D:I	Digestate	Microirrigation	Fallow	Fallow
NoCC:C:I	Compost	Microirrigation	Fallow	Fallow
CC-S:C:I	Compost	Microirrigation	White mustard	Ryegrass
CC-S:D:I	Digestate	Microirrigation	White mustard	Ryegrass
NoCC:D:R	Digestate	Rainfed	Fallow	Fallow
NoCC:C:R	Compost	Rainfed	Fallow	Fallow
CC-F:C:R	Compost	Rainfed	Triticale	Triticale
CC-F:D:R	Digestate	Rainfed	Triticale	Triticale

Table 3 Compositions of the compost and digestate matrices each year

Years	Organic matrices	Dry Matter (%)	Corg (%dm)	N (%dm)	P (%dm)	K (%dm)
1st	Compost	80.0	24	1.9	0.93	2.12
	Digestate	29.2	53.4	2.4	1.39	2.43
2nd	Compost	71.0	29	2.1	0.43	2.11
	Digestate	23.1	52.8	2.9	1.02	3.45
3rd	Compost	75.0	23	1.9	0.64	2.03
	Digestate	20.5	52.8	3.2	0.59	2.22
4th	Compost	55.4	31	1.93	0.58	2.10
	Digestate	20.2	52.8	3.2	0.61	1.74

treatment for maize in 2019 and soybean in 2020, and a pre-emergence treatment for maize 2021 and soybean 2022. The main compositions of the compost and digestate are reported in Table 3, and irrigation and fertilization management are summarized in Tables 4 and 5.

The winter CCs were sown using a sod-seeding drill on November 5th 2020 and October 18th 2021 and terminated with a rotary mulcher on March 27th 2021 and April 8th 2022. Triticale (var. Titania) was sown with a seeding rate of 204 kg ha^{-1} in both years, whereas white mustard (var. Maryna) in 2020 and ryegrass (var. Suxyl) in 2021 were sown at 29 and 63 kg ha^{-1} seeding rates, respectively.

Data collection

All the data collections described from here on were carried out in collaboration among researchers, farmers and technicians from CBVO.

Table 4 Number of drip irrigation events and total amount of water (mm) applied each year

Years	Number of irrigation events	Total water applied (mm)
2019	4	77
2020	3	51
2021	10	173
2022	20	157

Cash crop (total aerial biomass and grain) and CC biomasses (including the weeds present within the CC biomass samples) were sampled each year at harvest and termination time in 3 georeferenced sampling points of 4 m^2 for each plot. The dry matter content was determined by drying the biomass in a thermo-ventilated oven at 65 °C until constant weight was registered. CC dried biomass was chopped and analyzed for its C content (only for CCs) using a CNS

Table 5 Average organic carbon (Corg), N, P_2O_5, and K_2O supplied by the organic matrices throughout the 4 years of the experimentation

Organic matrices	Corg (Mg ha^{-1} y^{-1})	N (kg ha^{-1} y^{-1})	P (kg ha^{-1} y^{-1})	K (kg ha^{-1} y^{-1})
Compost	4.9	369	128	291
Digestate	5.2	328*	79	137

*In the first year, 120 kg ha^{-1} were supplied as mineral N to reach the maize request while maintaining the same Corg supply from compost and digestate

analyzer (elemental analyzer Vario Max, Elementar Americas, Inc., DE). Fixed C was determined by multiplying its concentration for dry biomass produced per unit area. The protein contents of the maize and soybean grains were determined by near-infrared spectroscopy (NIRS) (Infratec-1241 instrumentation, Foss Analytical, Hillerød, Denmark).

Soil samples (0–20 and 20–40 cm depths) were collected with a drill at the beginning (March 2019) and at the end (November 2022) of the experiment, and then left to be air-dried outdoors in boxes for about 1 month. Each soil sample was composed by 4 subsamples (one for each square meter). The dried samples were sifted to 2 mm and analyzed for their SOC content using a CNS elemental analyzer (Vario Max, Elementar Americas, Inc., DE).

Organic carbon balance

The OC balance was estimated in the first 0–40 cm of the soil layer. The SOC stock variation was determined as follows:

$$\mathrm{SOC_{stock}}\left(\mathrm{Mg\ ha^{-1}}\right) = \left[\mathrm{SOC_{nov22}}(\%) \times \mathrm{BD}\left(\mathrm{g\ cm^{-3}}\right) \times \mathrm{depth\ (cm)} \times 0.1\right] - \left[\mathrm{SOC_{mar19}}\ (\%) \times \mathrm{BD}\left(\mathrm{g\ cm^{-3}}\right) \times \mathrm{depth\ (cm)} \times 0.1\right]$$

where $\mathrm{SOC\%_{nov22}}$ and $\mathrm{SOC\%_{mar19}}$ are the percentages of SOC determined in the soils sampled in November 2022 and March 2019, respectively (see paragraph 2.3), BD is the soil bulk density determined according to Rawls et al. (1992), and 'depth' is the monitored 0–40 cm soil layer.

The exogenous OC (from compost and digestate) was calculated considering the compost and digestate composition (Table 3) and the supplied quantity (Table 5). The endogenous OC from aboveground cash crop residues and belowground biomass production, including rhizodeposition, was estimated on the basis of total aboveground biomass at harvest time. Aboveground residue dry matter was evaluated at harvest time. Belowground biomass production was estimated to be 1.1 times and 0.2 times the dry matter residues of maize (Dal Ferro et al. 2020) and soybean (Nissen et al. 2008), respectively. The crop residues and root C content was estimated to be 45% of dry matter (Kätterer et al. 2011). Aboveground CC dry matter was measured at harvest time, and its C content was measured as reported in paragraph 2.3. On the basis of a previous experiment (data not shown), CC belowground biomass production was estimated to be 2.1, 1.3, and 1.1 times the aboveground dry matter of triticale and ryegrass, weeds, and white mustard, respectively. The C content of the belowground biomass was estimated to be 45% of the dry matter (Kätterer et al. 2011). The efficacy of organic C fixation was calculated as the ratio between the SOC stock variation and total organic C inputs (exogenous C + endogenous C).

Statistical analysis

Considering the variability of the physico-chemical parameters of the samples taken in the rainfed area of the farm versus the ones taken in the irrigated area before the beginning of the experiment (Table 1), the soil dataset was split in two subsets to avoid masking possible effects of the practices implemented in the experiment. Three permanent plots of 4 m^2 each were established for each of the ten combinations of treatments tested on the farm; they were distributed along a longitudinal transect at regular intervals from the field borders and between two consecutive fields. Each permanent plot was identified with the only purpose of sampling but was managed with the same field operation occurring in the relative field.

Statistical analyses of the above-listed variables were performed using RStudio software (Core Team R 2014). All the outcome variables were analyzed using linear models where the CC treatment, fertilization, irrigation and their interaction were used as fixed factors. Marginal and conditional residual distributions were checked visually to detect possible issues of non-normality or heterogeneity of variances. An analysis of variance (ANOVA) of each model was performed and the Tukey's HSD test at $P < 0.05$ was used as post-doc analysis.

Results

Meteorological data

Yearly rainfall was above the 30-year average (996 mm) in the first year (2019, +206 mm), whereas it was lower in 2020 (−222 mm) and 2022 (−354 mm). The distribution of rainfall in 2019 showed high precipitation events concentrated in spring (249 mm in April and May on average) and the winter months of November and December (177 mm on average) (Fig. 3). In 2020, high precipitation were observed in June (206 mm), in September and October (132.9 mm on average), and in December (147 mm), after CC sowing. A similar precipitation distribution to 2019 was observed in 2021, when high precipitation events were recorded in spring (122 mm in April and May on average) right after CC termination, and in November (162 mm) after CC sowing. In 2022, the highest value of 138 mm was recorded in September, while an average of 113 mm was measured in November and December. The highest and lowest air temperatures were measured in July and January, respectively, confirming the pattern observed in the last 30 years. However, the yearly average maximum (19.9 °C) and minimum (9.4 °C) temperatures recorded during the experimental period were + 4.2% and + 5.6% higher than the average 30-year values, respectively. The distribution of monthly cumulative ET0 showed the lowest values from November to January (9.1 mm month^{-1} on average) throughout the 4 years, while the highest value was from June to August (137 mm month^{-1} on average).

Crop growth and grain quality

The crop aboveground biomass was significantly affected by the fertilizer in three out of four years (Table 6). Digestate application increased the aboveground biomass of maize in both growing seasons (+26.0% and + 37.7%, in 2019 and 2021, respectively) compared with compost (6.05 ± 0.19 Mg ha^{-1} and 6.72 ± 0.60 Mg ha^{-1}, respectively). The same effect was observed for grain yield (+88.2% and + 37.4%, in 2019 and 2021, respectively) compared with compost application (4.91 ± 0.24 Mg ha^{-1} and 4.07 ± 0.48 Mg ha^{-1}, respectively). The digestate significantly increased (+29.9%) the aboveground

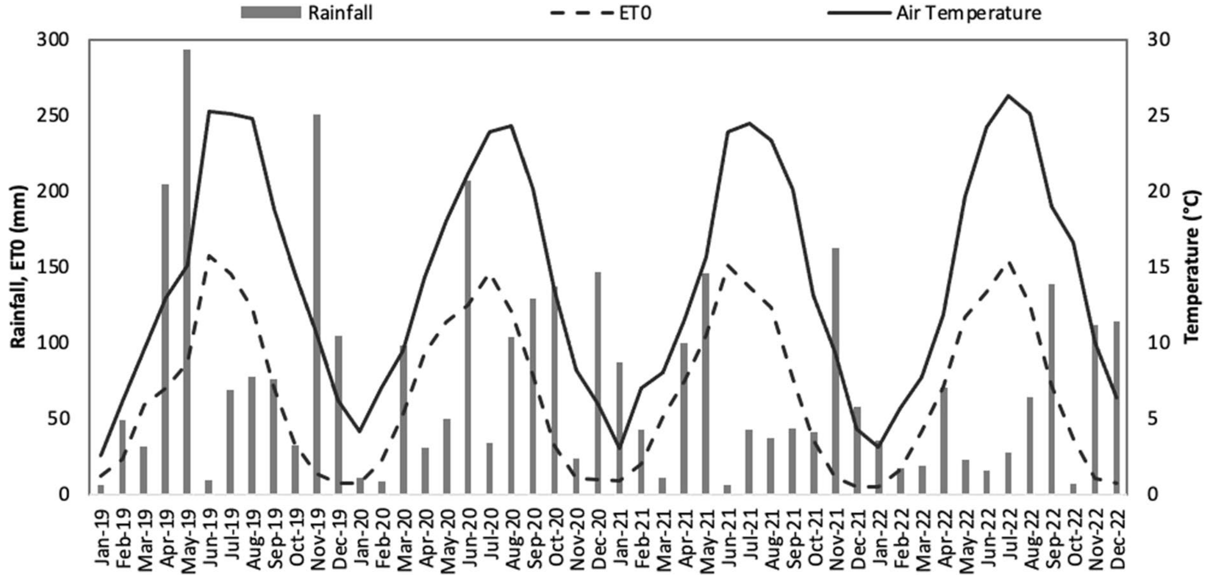

Fig. 3 Monthly rainfall, evapotranspiration (ET0) and mean air temperatures from 2019 to 2022 in Noventa di Piave (5 km from San Donà di Piave)

Table 6 ANOVA significance table showing the effects of fertilization, irrigation, and cover crop presence and type on maize and soybean growth and agronomic parameters

Source of variation	AB	GY	GP
Maize 2019			
F	**0.0009**	**<0.0001**	**0.0323**
I	0.6182	0.4880	0.2253
F x I	0.5391	0.7918	0.4172
Soybean 2020			
F	0.2112	0.4271	0.1693
I	0.1119	0.2398	**0.0034**
F x I	0.7844	0.1519	0.1238
Maize 2021			
CC	**0.0047**	**0.0268**	0.6697
F	**0.0101**	**0.0409**	0.6027
I	**0.0068**	**0.0018**	0.5391
CC x F	**0.0393**	0.2552	0.3257
CC x I	**0.0244**	**0.0356**	0.3726
F x I	0.0747	0.0824	0.6027
CC x F x I	0.3101	0.3565	0.8123
CC*	0.5041	0.7015	0.3879
F	0.5436	0.9904	0.9112
CC* x F	0.7388	0.9018	0.8466
Soybean 2022			
CC	0.3909	0.5168	0.2476
F	**0.0054**	0.7461	0.1236
I	**<0.0001**	**<0.0001**	0.2150
CC x F	0.4142	0.8600	0.1769
CC x I	0.9096	0.5703	0.3679
F x I	0.0529	0.0912	0.7766
CC x F x I	0.8951	0.4328	0.0717
CC*	0.2476	0.9118	0.1468
F	**0.0011**	0.7863	0.0962
CC* x F	0.4707	0.4285	0.7739

Significant p-values are reported in bold

AB aboveground biomass, *GY* grain yield, *GP* grain protein content, *F* fertilization (compost vs. digetate), *I* Irrigation (microirrigation vs. rainfed), *CC* cover crops (NoCC vs. CC-F), *CC** cover crops (NoCC vs. CC-F vs CC-S)

biomass of soybean compared with compost (2.88 ± 0.24 Mg ha^{-1}) only in the 4th year, but the fertilization treatment never influenced grain production (4.65 ± 0.12 Mg ha^{-1} and 2.05 ± 0.18 Mg ha^{-1} on average in 2020 and 2022, respectively).

Irrigation had a significant effect only in the 3rd and 4th years of the experiment, with opposite trends. In the 3rd year, maize aboveground biomass and grain yield were significantly higher under rainfed conditions than under irrigation (6.1 ± 0.8 Mg ha^{-1} vs. 3.8 ± 0.4 Mg ha^{-1}). Conversely, the aboveground biomass of soybean was significantly improved by irrigation compared with the rainfed condition in the 4th year (2.7 ± 0.1 Mg ha^{-1} vs. 1.0 ± 0.1 Mg ha^{-1}).

Considering the effect of management on grain yield in the 4 years, the integrated practices that involved digestate outperformed those based on compost, except when compost was used under irrigation and in the presence of CCs (Fig. 4). The introduction of irrigation in agronomic management had a significant effect in all 4 years only in the field under compost fertilization and CC-F (+16.2% for cumulative grain production).

The grain protein content was not significantly affected by the different factors throughout the experiment, except maize fertilization in 2019 (7.81 ± 0.14% and 8.23 ± 0.12% with compost and digestate, respectively) and soybean irrigation in 2020 (42.69 ± 0.14% and 43.33 ± 0.14% under irrigated and rainfed conditions, respectively).

Soil organic carbon

Soil organic carbon under irrigation management

Under irrigation management, the year, the CC, the fertilization x CC interaction and the year x fertilization interaction significantly affected the SOC content. In addition, SOC was significantly affected by the year x sampling depth x fertilization interaction (Table S.1). The highest increase in SOC was recorded in the topsoil layer (0–20 cm) under compost fertilization (+35.7%), then in the deeper soil layer (20–40 cm) (+18.5%) under the same management. Under digestate treatment, the SOC increase was not consistently different from the starting condition (Fig. 5).

Soil organic carbon under rainfed management

Under rainfed conditions, the OC content was significantly affected by the year, the CC, the year x CC x fertilization interaction and the CC x sampling depth x fertilization interaction (Table S.2). As for the effect of the year x CC x fertilization interaction on the OC content, the highest value was recorded at the end of the experiment under compost fertilization in the absence of a CC. At the end of the experiment, the OC content was almost similar in

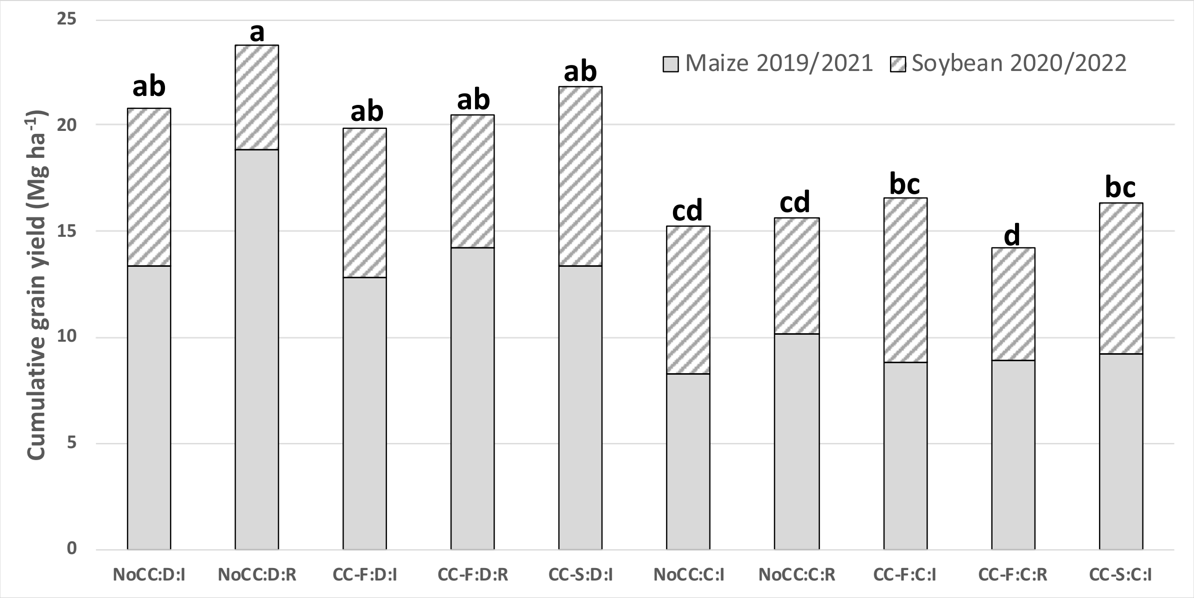

Fig. 4 Four-year cumulative grain yield (Mg ha^{-1}) following the ten tested management practices, and common district yield as a comparison. Different letters indicate significant differences at $p < 0.05$ (Tukey HSD test)

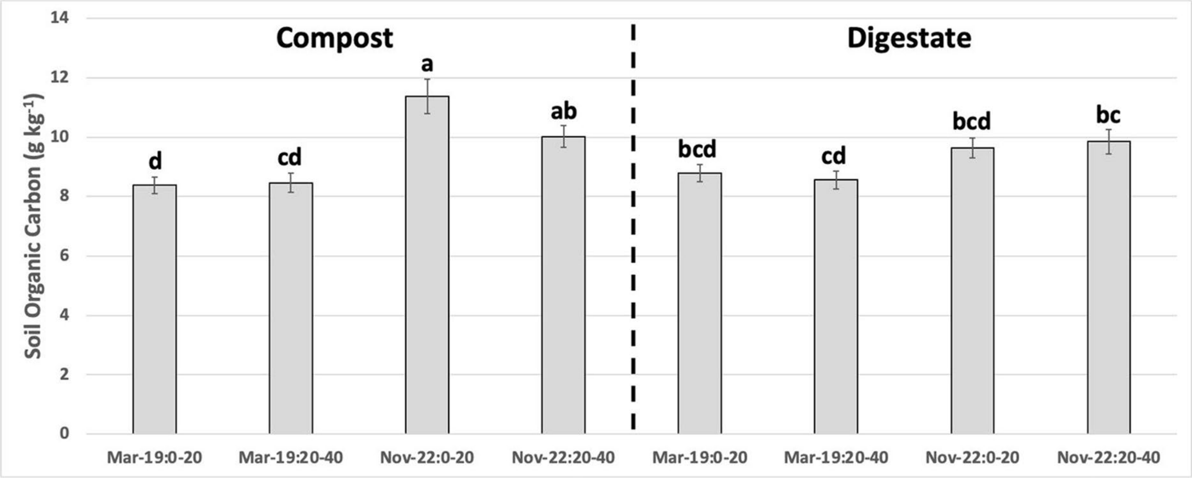

Fig. 5 Soil organic carbon content (g kg^{-1}) with the two organic fertilizers at different times of the experiment (beginning: Mar-19; end: Nov-22), in soil sampled at two depths (topsoil: 0–20; deep soil: 20–40 cm) under irrigation management. Different letters indicate significant differences at $p < 0.05$ (Tukey HSD test). Vertical bars, standard errors

all the other CC and fertilization combinations and close to the content found at the beginning of the experiment. The only consistent improvement was found under compost fertilization in the plot without a CC and under digestate fertilization with CC-F (Fig. 6).

Carbon budget

The cumulative OC input was significantly different among the ten integrated practices due to different endogenous OCs (Table 7). The exogenous OC supplied by organic fertilization was similar in

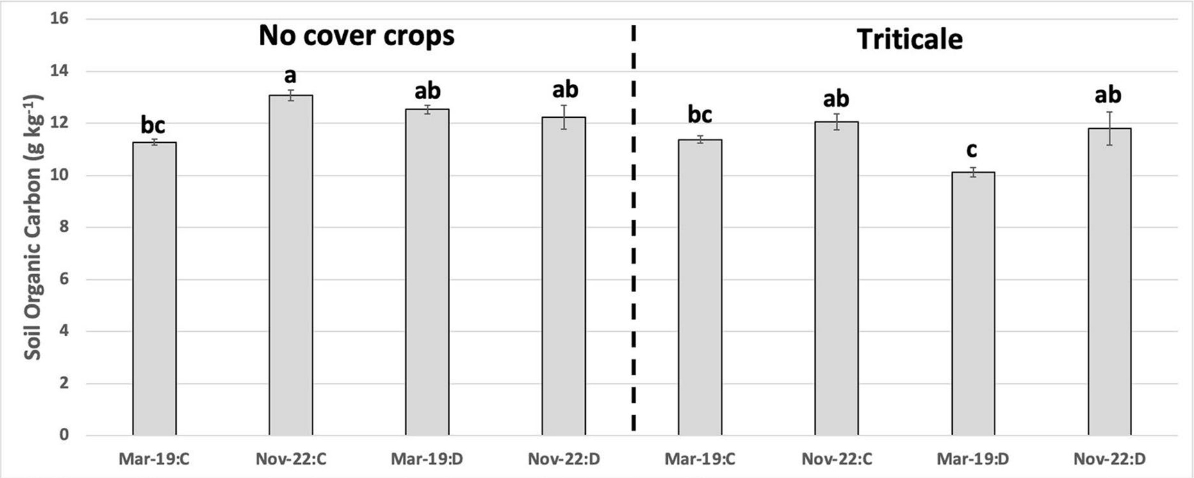

Fig. 6 Soil organic carbon content (g kg^{-1}) under rainfed management with different cover crop management practices at different times (beginning: Mar-19; end: Nov-22) and with the two organic fertilizers (digestate: **D**; compost: **C**). Different letters indicate significant differences at $p < 0.05$ (Tukey HSD test). Vertical bars, standard errors

the compost (19.4 ± 1.0 Mg ha^{-1}) and the digestate (20.9 ± 1.4 Mg ha^{-1}), whereas different cash crop residue and CC biomasses were measured among the integrated practices (Table 7). Exogenous OC represented 44.5% (NoCC:D:R) to 56.1% (CC-F:C:R) of the cumulative OC input. After 4 years, the SOC stock increase ranged from −1.2 Mg ha^{-1} (No-CC:D:R) to 10.3 Mg ha^{-1} (CC-S:C:I), corresponding to OC fixations of −2.9% and + 27.8%, respectively. Considering the main effects combined in the ten integrated practices, it was observed in the short term that: i) irrigation significantly increased the SOC stock (+7.2 ± 0.7 Mg ha^{-1}) compared with the rainfed condition (+ 4.0 ± 1.2 Mg ha^{-1}); ii) cash crop fertilization with compost significantly increased the SOC stock (+7.8 ± 0.8 Mg ha^{-1}) compared with digestate (+ 4.0 ± 0.9 Mg ha^{-1}); iii) the introduction of CCs and their management did not have any effect

Table 7 Changes in SOC stock and fixed organic carbon (OC) measured under different integrated practices. Estimated SOC accumulation rates based on the "4 per 1000" initiative in the topsoil (0–40 cm)

Treatment	Exogenous OC (Mg ha^{-1})	Endogenous OC (Mg ha^{-1})				Cumulative supplied OC (Mg ha^{-1}) (1)		ΔSOC stock (0–40 cm) (Mg ha^{-1}) (2)		Fixed OC (1:2 ratio) (%)		*"4 per 1000" check* (Mg ha^{-1})
		Cash crops		Cover crops								
	2019–2022	2019–2022		2021–2022		2019–2022		2019–2022		2019–2022		2019–2022
CC-F:D:I	21.5	17.4	ab	2.5	ab	39.6	ab	5.7	abc	13.9	ab	0.613
CC-F:C:I	19.7	15.0	b	2.1	bc	35.3	b	9.3	a	25.1	a	0.675
NoCC:D:I	20.4	19.2	ab	1.4	bc	39.9	ab	4.7	abc	12.0	b	0.622
NoCC:C:I	18.8	14.9	b	0.2	c	33.8	b	9.4	a	27.6	a	0.569
CC-S:C:I	20.9	16.8	ab	1.3	bc	38.1	ab	10.3	a	27.8	a	0.638
CC-S:D:I	20.2	18.5	ab	1.6	bc	39.2	ab	3.5	bcd	9.1	bc	0.699
NoCC:D:R	19.3	23.8	a	1.4	bc	43.4	a	−1.2	d	−2.9	c	0.904
NoCC:C:R	18.4	16.4	b	1.6	bc	35.2	b	7.3	abc	21.0	ab	0.822
CC-F:C:R	19.3	14.3	b	2.9	ab	34.5	b	2.8	cd	8.1	bc	0.829
CC-F:D:R	23.0	19.0	ab	4.3	a	43.2	a	7.0	abc	15.1	ab	0.745

CC-F fixed treatment (X *triticosecale*), *CC-S* succession treatment (*Sinapis alba* L.; *Lolium multiflorum* Lam.), *NoCC* no cover crop, *D* digestate, *C* compost, *I* drip irrigation, *R* rainfed on the SOC stock. All integrated practices but the NoCC:D:R condition reached the "*4 per mille*" goal.

Discussion

Soil organic carbon

Among the tested practices, compost addition under irrigation management contributed to the most consistent SOC content increase in the topsoil (0–20 cm depth) compared to digestate. This can be mainly explained by the different contributions of the different materials and ensuing organic matter stability levels. Compared with compost addition, digestate addition to the soil provided easier available organic matter, mostly degradable in the short term (Alburquerque et al. 2012). Martínez-Blanco et al. (2013) showed that C sequestration following compost application was higher in the short term (up to 40% of the applied C) and decreased down to 2–16% over a 100-year period. In our study, average C sequestration following compost addition was 21.9% after 4 years.

Irrigation improved the SOC stock in the 0–40 cm layer, in accordance with Emde et al. (2021) on the basis of 47 case studies located all over the world. The CCs were introduced only in the second two-year period of experiment. They contributed to increase the SOC content depending on the CC species and the quality of its biomass (e.g., C:N ratio), and returned to the soil more than the sole cover crop C (Higashi et al. 2014; Sias et al. 2021). As already reported by other authors (Jian et al. 2020; Qin et al. 2023), although the CC-F condition returned 2.1 times more C to the soil than the other conditions, its contribution in terms of SOC storage in the first 0–40 cm was only 89% that of the CC-S condition. However, we did not observe a consistent effect of CCs on the SOC stock increase in the first 0–40 cm soil layer. This confirms that the time since CC introduction (Poeplau and Don 2015) and CC biomass production (Duval et al. 2016) are key aspects of SOC stock changes.

In order to meet the aim of many of the farmers of the area where the on-farm experimentation was implemented, that is maximizing the SOC stock within the shortest possible period, the most promising management strategy should integrate fertilization with compost and irrigation independently from the adoption of CCs during the fallow period.

Side effects

Although the aim of the study in terms of SOC stock increase was reached, the short-term sustainability of the grain yield of both cash crops throughout the 4 years of the experiment should be considered.

From a merely productive perspective, the strategy solely based on organic fertilization led to an overall maize grain yield loss compared to the production level that can be attained in the same rural district in farms adopting adequate fertilization based on chemical inputs (5.9 Mg ha^{-1} in this study vs. 12.7 Mg ha^{-1} on average in 4 farms). This result can be attributed to the mismatch between N release from organic fertilizers and N uptake by maize, especially when compost is used, and could deter farmers from using these sources of fertilization. A possible solution might be mixed fertilization to combine the targets of soil organic matter increase and satisfactory yields (Maucieri et al. 2019). Contrary to maize, the soybean grain yield was similar to the production level of the farms of the district (3.4 Mg ha^{-1} in this study vs. 3.1 Mg ha^{-1} on average in 7 farms) (Table S.3) due to its capability to fix N. In addition to yield loss, costs related to organic fertilization must be considered. Indeed, adding costs for purchase, transport and distribution, organic fertilization was more expensive than the mineral one (+12% for compost and + 20% for digestate).

The higher productivity of maize under digestate fertilization can be explained by the different natures of the two organic fertilizers (Tambone et al. 2010), and different mineralization rates – a variable that can determine different amounts of N release during the cash crop growing cycle (Di Mola et al. 2021; Farneselli et al. 2022; Tambone and Adani 2017; Zaccardelli et al. 2021;). In addition, the highest grain yield recorded in the 1st year of the experiment could be related to the additional amount of N supplied through urea, in order to align the quantity of N supplied in the field managed with the two different organic fertilizers to maintain the supplied OC constant.

Irrigation significantly supported the cumulated crop grain yield when combined with compost fertilization and CC-F with triticale. Conversely, irrigation did not have the same effect on crop production under digestate fertilization combined with the same CCs (CC-F). Those results could be explained by the stimulation effect of irrigation on soil microbial activity and soil organic matter mineralization when less N is available.

Conclusions

An on-farm experimentation was co-developed with local farmers and a land reclamation authority to answer their specific aim of increasing SOC within a short time. Almost all strategies increased the SOC content and stock. Considering the results, the most promising management strategy should integrate organic fertilization with compost and crop irrigation, independently from CCs during the fallow period. However, the potential of CCs to enhance the SOC stock should be further investigated, as previous studies have reported promising long-term results.

Although the SOC stock increase within a short period was achieved, a question still remains open about the lower productivity of maize when solely fertilized with organic sources of N.

Our findings suggest that SOC accumulation is achievable in the short term with abundant applications of organic biomass, but the strategy might lead to economic losses (lower maize productivity).

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Supplementary material

Table S.1 - ANOVA significance table showing the effects of the year, the cover crop, the sampling depth and the fertilization type on soil organic carbon under irrigation management.

Source of variation	Organic carbon
Year (Y)	**2.09e-09**
Cover (C)	**0.0013**
Depth (D)	0.1670
Fertilization (F)	0.1278
Y x C	0.9666
Y x D	0.2830
C x D	0.1184
Y x F	**0.0109**
C x F	**0.0132**
D x F	0.1744
Y x C x D	0.2774
Y x C x F	0.8074
Y x D x F	**0.0471**
C x D x F	0.1527
Y x C x D x F	0.24360

Table S.2 - ANOVA significance table showing the effects of the year, the cover crop, the sampling depth, and the fertilization type on soil organic carbon under rainfed management.

Source of variation	Organic carbon
Year (Y)	**3.020e-05**
Cover (C)	**4.351e-05**
Depth (D)	0.6770
Fertilization (F)	0.1813
Y x C	0.2917
Y x D	0.8348
C x D	0.5328
Y x F	0.1813
C x F	**0.0215**
D x F	0.1880
Y x C x D	0.0520
Y x C x F	**0.0005**
Y x D x F	0.0944
C x D x F	**0.0138**
Y x C x D x F	0.0568

Table S.3 - Maize and soybean grain yields (Mg ha^{-1}) in farms of the same area during their respective cropping seasons (average ± SD).

Crop	**Year**	**Grain yield (Mg ha^{-1})**
Maize (4 farms)	2019	126.1 ± 15.3
	2021	128.4 ± 11.7
Soybean (7 farms)	2020	37.5 ± 5.6
	2022	24.6 ± 8.0

Conclusions

It is important to contextualize the findings delineated in this thesis within the framework of an experimental period spanning three to four years subsequent to the initial introduction of the CCs. While acknowledging that this timeframe might not afford ample opportunity for cover crops (CCs) to elicit permanent alterations in the biochemical components of nitrogen (N) cycling, soil water content (SWC), and Soil Organic Carbon (SOC) accumulation, the findings do, however, offer insights into prevailing trends. These insights are valuable as they shed light on the potential and limitations of incorporating CCs in the short term. This perspective holds particular relevance for farmers hesitant to adopt CCs in their agricultural systems due to concerns about potential short-term adverse effects that might impact the economic viability of this practice.

We understood that maximizing the advantageous effects of CCs on biochemical N dynamics and the SWC of agricultural systems necessitates a deep understanding of their growth pattern, biomass production, N accumulation, and subsequent decomposition of residues involving mineralization-immobilization processes. These aspects are highly influenced by a multitude of factors such as biotic elements, pedo-climatic conditions, and management practices.

The research studies reported in the present thesis revealed the following effects of the CCs introduction in cropping systems.

- *Cash crop yield.* When CCs are introduced into conventional farming systems, without management alteration from the 'business as usual', they can sustain comparable yields of maize and soybean compared to fallow controls without weed control measures.
- *Biochemical N dynamics.* Diverse CCs differently affected both soil chemical (NO_3^-) and biological (soil N functional genes - NFGs) constituents of the N cycle across different phases of the crop rotation.

  Grass CCs (rye and triticale), provided soil coverage early in winter and reduced soil NO_3^- content (acting as catch crops) during their growth phase. They also potentially facilitated higher microbial-mediated N fixation compared to fallow control. The model CC-NCALC (for CCs biomass N release prediction) estimated a slow decomposition rate for both rye and triticale once their residues were incorporated into the soil. Both grass species indeed showed an immobilization of soil N resources, especially pronounced in the case of triticale, which persisted throughout the cash crop growing season. This immobilization resulted in reduced maize N uptake even when the yield was not affected showing similar values to control and clover CCs treatment. On the contrary, clover CCs may exhibit elevated residual soil NO_3^- upon CC termination and promote increased potential for microbial-mediated N nitrification activity. The residues of clover CCs tend to undergo rapid decomposition (CC-NCALC model), releasing a higher quantity of N compared to grasses residues. For this reason, in the context of managing N dynamics to avoid potential depletion of quantity and/or quality of yield, clover CCs might represent a preferable option during the initial years of CC introduction compared to rye and triticale CCs. Noteworthy was that mustard CCs, despite producing a biomass akin to that of grass species, it was estimated to release (CC-NCALC model) a similar N quantity as clover. However, it demonstrated a high susceptibility to winter climatic conditions. Yet, the potential for accelerated decomposition of its residues and subsequent N release is an aspect that warrants further scrutiny when trying to understand how to integrate standard N fertilization with N coming from CC residues.

In conventional farming systems, various agronomic practices have the potential to obscure the effects of CCs on the biochemical N dynamics. While the N fertilization of cash crops can prevent potential yield depletion following grass CCs species (likely attributed to N immobilization) they may also conceal the potential contribution of N resources derived from the decomposition of clover or mustard residues. Furthermore, tillage operations for seedbed preparation might disrupt differences in soil NFGs abundances after diverse CCs residue incorporation. Additionally, apart from the impacts associated with the introduction of CCs just reported, we observed that the choice of cash crop species itself can further reshape and alter microbially mediated N transformation processes during the cash crop growing season.

- *SOC accumulation.* The integration of CCs in a conventional cropping system did not significantly increase SOC content in the short term (when combined with the application of organic fertilization matrices).
- *SWC.* Despite the presence of CCs affecting the temporal variation of SWC, none of the CCs tested exhibited water competition with subsequent cash crops.

Concerning the monitoring approaches implemented for conducting the experimentations:

- *Remote sensing.* The utilization of remote sensing tools such as satellite images (from which derive VIs) enabled the reliable monitoring of CC growth patterns and highlighted distinct variations in soil coverage among CCs species during the winter season. Nonetheless, it is imperative to evaluate this approach across diverse CCs species, soil types, and varying climate conditions to furnish precise recommendations for CCs management. Further investigations are essential to assess the efficacy of alternative tools utilizing a broader spectrum of wavelengths or enhanced spectral resolution.

- *Crop samples*. Assessing the entire plant, encompassing root biomass, provided a dependable means of evaluating the quantity of residues incorporated into the soil. Failure to consider this component might have led to an underestimation of CCs residue biomass contribution by more than 50%.
- *Modeling approach for CCs N release.* Employing decomposition models, specifically CC-NCALC, allowed for the estimation of CC N contribution to subsequent cash crops, offering potentially valuable insights to enhance the efficacy of cash crop N fertilization management. However, as the CC-NCALC model constitutes a web-based tool developed in the United States intended for utilization by farmers, it will necessitate additional implementation to cater to various crops, environmental contexts, and diverse types of plant residues (such as those of the above and belowground biomass). These implementations are essential for leveraging the model's application in scientific research endeavors.
- *Biological indicators of N cycle.* NFGs proved to be sensitive indicators for evaluating the biochemical N cycle. However, their measurement can be influenced by various factors, posing challenges in isolating the primary effect under analysis. Hence, it is imperative to select an appropriate sampling time, especially in the case of CCs, which may be at the time of their termination, to ensure accurate and replicable assessments.

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