UNIVERSITÀ CATTOLICA DEL SACRO CUORE
Sede di Piacenza

Dottorato di ricerca per il Sistema Agro-alimentare
Ph.D. in Agro-Food System

Cycle XXXIV
S.S.D. AGR/02

Contribution of agroecological measures towards climate-smart and multifunctional farming systems

Candidate:

Federico Ardenti

Matriculation n:

4814709

Academic Year 2020/2021

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Coordinator:
Ch.mo Prof. Paolo Ajmone Marsan

Tutor: Prof. Vincenzo Tabaglio

Candidate:

Federico Ardenti

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SUMMARY

SUMMARY

Growing population, land degradation and climate change are significant threats to food security and human development. Agriculture impacts on the climate change by contributing to anthropogenic greenhouse gas (GHG) emission through carbon dioxide (CO_2), nitrous oxide (N_2O) and methane (CH_4). At the same time, agricultural activity is directly affected by unfavourable consequences of global warming with a detrimental effect on yields and soil quality, thus mining food security for a growing population. Food productions today are based on conventional intensive farming, which depleted soil quality and polluted the environment through soil organic matter (SOM) mineralization as CO_2, nitrogen (N) release as reactive forms (N_2O, NO_3^-, NH_3) to atmosphere and water, and phosphorus (P) accumulation and runoff.

The adoption of sustainable agroecosystems management is key for adapting to climate change while lowering agricultural impact on global warming. Resilient practices can lead to preserve existing C stocks and to remove at the same time C from the atmosphere, while having a positive impact on food security, agro-industries, water quality and the environment. Following Conservation Agriculture (CA) practices is widely indicated as a recommended way to pursue those objectives. Besides C sequestration into the soil, also N_2O emissions reduction must be taken into account when planning to develop practices for climate change mitigation. Using high-efficient fertilization and irrigation systems is a viable strategy to increase resource use efficiency, thus limiting environmental impact of agriculture. In particular micro-irrigation systems, combined with fertigation techniques, are suggested as a measure to reduce N_2O emissions from soils and increase N-use efficiency of crops.

The specific objectives of this thesis are: (1) to evaluate the yield performances of climate-smart practices (i.e., no-till, cover crops, and subsurface drip irrigation) on maize, soybean, and winter wheat as compared with conventional agriculture practices (moldboard plowing without cover crops and sprinkler irrigation); (2) to assess the potential of no-till and cover crops to provide biomass, C, and N input to the soil, and how these practices may affect SOC and STN dynamics and accumulation in the short- and long-term; (3) to measure the effect of contrasting irrigation/fertilization systems (subsurface drip irrigation + fertigation vs. sprinkler irrigation + granular application of fertilizers) on N_2O emissions and N use efficiency of maize and soybean.

The thesis has 3 research chapters, next to a general introduction (Chapter 1) and a general discussion (Chapter 5).

In Chapter 2, we (i) examined how different widespread CCs (i.e. rye; phacelia + white mustard ; italian ryegrass + crimson clover + persian clover; hairy vetch + crimson clover) affect in the short-term yield performance of main crops under NT as compared with no-CCs, during a 3-year crop sequence (i.e. maize, soybean, and maize); (ii) assessed the 3-year effects of CCs treatments on inputs (i.e. biomass, C, and N) to the soil, SOC and C pools concentrations, as well as soil fauna (i.e. microarthropods and earthworms) abundance and diversity. We found that grain yield during the initial 2-yr period was on average reduced with CCs by 1-23% in maize, and 1-33% in soybean. This effect was less evident with CC residues having low C:N ratio (< 20; i.e. hairy vetch + crimson clover) and erects posture after termination (i.e. rye). Thereafter, CCs had no effect on maize yield the third year. In addition, the results on soil organic C and pools indicated that (i) the effect of our CC treatments over a 3-yr application is limited to the topmost 5 cm of soil, and (ii) the biomass input with CC residue and its C:N ratio are crucial for boosting soil C cycling in the short-term.

In Chapter 3, we investigated the long-term effect of no-till (NT) coupled with a grass rather than a legume cover crop (i.e., rye [NT-R] and hairy vetch [NT-V]) on main crop yields, biomass input by cover and main crops, soil aggregation and C and N sequestration rates, in comparison with conventional tillage (CT). The study, lasting nine years, was performed on a winter wheat-maize-soybean crop rotation. We found that yield of winter wheat, maize, and soybean were never reduced under both NT treatments, neither during the transition phase, nor afterwards. Rye and hairy vetch provided the same amount of biomass and C input, although vetch doubled N input compared with rye. Moreover NT-V increased cumulative biomass and C input from main crop residues compared with NT-R. Both NT-R and NT-V promoted C (+0.4 Mg ha^{-1} y^{-1} and +0.6 Mg ha^{-1} y^{-1}, respectively) and N (+88 kg ha^{-1} y^{-1} and +145 kg ha^{-1} y^{-1}, respectively) soil sequestration, mainly due to the increase of macroaggregate-associated C and N, thus corroborating a major role of NT for macroaggregates formation and SOM stabilization within macroaggregates.

In Chapter 4, we evaluated the effect of three irrigation systems (subsurface drip irrigation with a narrow dripline spacing [dripline distance of 70 cm; SDI70] vs. subsurface drip irrigation with a wide dripline spacing [dripline distance of 140 cm; SDI140], vs sprinkler irrigation [SPR]) on yield, N_2O emissions and N-use efficiency of maize and soybean. We found that SDI may increase maize yield (+31%) and N-fertilizer efficiency (+43-

71%). These positive results were only observed during the drier year in which irrigation supplied ca. 80% of maize water requirements. The narrower dripline spacing mitigated N_2O emissions compared with sprinkler irrigation (by 44%) and with the wider spacing (by 36%), due to a more homogeneous distribution of N in soil, and to a lower soil moisture content. Soybean yield and N-use efficiency were not affected by the irrigation systems.

To resume, main conclusions of this PhD thesis are as follows:

i. Conservation practices (no-till and cover crops) may ensure comparable crop yield to conventional systems especially in the long-term (Chapters 2 and 3), while high-efficient micro-irrigation systems (subsurface drip irrigation) are particularly effective to boost crop yield under dry climate conditions (Chapter 4);
ii. Particular attention should be paid when pairing no-till with high C:N ratio cover crops because of the negative effect on yield due to reduced soil temperatures and increased N immobilization (Chapters 2 and 3);
iii. Large return of fresh organic material through cover cropping and avoiding soil disturbance through no-till may increase C sequestration into the soil by boosting humification processes, stimulating macroaggregate production and physically protecting SOM within aggregates (Chapters 2 and 3);
iv. Labile forms of C scarcely contributed to SOC accumulation in the short-term under no-till, whereas it greatly contributed in the long-term through cPOM protection within macroaggregates (Chapters 2 and 3);
v. The effect of conservation practices on C sequestration was affected by residue biomass quality (C:N ratio) in the short-term, while biomass production rate over residue quality was the main driver for SOC accumulation in the long-term (Chapters 2 and 3);
vi. N accumulation into the soil was not affected by diverse CC species (legumes *vs.* grasses) probably due to N susceptibility to losses via NO_3^- leaching and/or N_2O emission (Chapters 3 and 4);
vii. SDI is a promising irrigation system of which benefits (N use efficiency increase and N_2O emissions reduction) are particularly significant when dripline distance matches plant spacing and when used under dry climate conditions (Chapter 4).

CHAPTER 1.

General introduction

1.1 Relationship between agriculture and global climate change

Global climate change is a major challenge for human development in the next future (Mora *et al.*, 2018), threatening resource availability, crop productivity and, therefore, food security. In fact, higher air and earth temperatures have dramatically reduced water availability in many world areas, due to increased evapotranspiration and prolonged periods of rain water shortage (Abtew and Melesse, 2013). This is particularly critical in dry climates, but even in temperate areas climate is recently heading towards hotter and drier summer seasons (Brown, 2020), increasing the risk of prolonged droughts and reduced yields. In addition, climate change has negatively affected soil fertility by reducing soil nutrient storage because higher soil and air temperatures are known to promote nutrient mineralization and losses (Kirschbaum, 2000).

To further complicate matters, agriculture is not just affected by global warming, but it is among the main causes of climate change by contributing to 23% of the total anthropogenic greenhouse gas (GHG) emission (Shukla *et al.*, 2019). The impact of agriculture on the environment is often linked with the so called "conventional farming practices", which originated with the green revolution and are based on intensive soil tillage operations, crop specialization, and large use of external inputs (i.e., mineral fertilizers, herbicides, pesticides, and fossil fuels) (Aziz *et al.*, 2013). Intensive soil tillage operations, such as moldboard plowing and rotary harrowing, are extensively used for seedbed preparation and weed control (Larney and Fortune, 1986). However, several studies highlight that tillage operations cause physical disturbance to soil, thus promoting soil aggregates disruption and exposing organic residues to the activity of soil microorganisms (Six *et al.*, 2000b; Kladivko, 2001; Conant *et al.*, 2007; Perego *et al.*, 2019). This results in increased carbon (C) losses from soil via carbon dioxide (CO_2) emissions, enhancing agricultural impact on global warming (Paustian *et al.*, 2000). Moreover, tillage operations promote itself CO_2 emissions from agricultural activities due to the use of fossil fuels. Besides tillage practices, the massive use of nitrogen (N) fertilizers in conventional farming systems has been indicated as one of the major causes of environmental pollution (Byrnes, 1990). In fact, since the development of the Haber-Bosch process, nitrogen (N) fertilizers are extensively (over)used to address N deficiency in crops, leading to inefficiencies and significant N losses especially when N inputs exceed plant needs or soil system capacity (Gruber and Galloway, 2008). N losses from soil may cause water contamination through nitrates (NO_3^-) percolation and promote climate change through nitrous oxide (N_2O) emissions (Byrnes, 1990).

Therefore, understanding C and N dynamics into the soil is a key aspect for developing climate-smart farming strategies aimed to reduce environmental impact of agriculture. In particular, the following chapters will focus on the importance of soil C storage and N_2O emissions reduction for revising farming practices towards an ecological intensification of agro-ecosystems.

1.1.1 Soil C storage and C pools

Soil can act as either a source or a sink for C (Fearnside and Barbosa, 1998). In detail, the potential of soil for long-term C sequestration (and therefore for GHGs emission mitigation) has been estimated to range from 0.2 to 1.87 Pg C per year depending on land use and location (Lal and Bruce, 1999; Conant *et al.*, 2001; Rattan Lal, 2008). Soil C levels are ultimately determined by the ratio between C inflows, for example via belowground allocation of photosynthesis, and outflows such as for organic matter decomposition (Amundson, 2001). Hence, soil C content variation may have an important impact on the global C cycle (Raich and Potter, 1995). Carbon compounds into the soil can be distinguished as either organic or inorganic. Soil inorganic C is one of the main constituents of carbonate minerals, which originate from weathering of parent material, or from reaction of soil minerals with atmospheric carbon dioxide (CO_2). In contrast, soil organic carbon (SOC) is one of the main components of soil organic matter (SOM). SOC includes relatively available C as fresh plant residue or living organisms and relatively inert C in materials derived from plant remains: humic substances and charcoal (Lal, 2006).

Variation in soil carbon stocks is primarily governed by two fundamental factors: (i) input of fresh organic matter (OM) and (ii) its decomposition rate (Kong *et al.*, 2005; Lützow *et al.*, 2006). Moreover, land use and management, which affects soil bio-chemical properties and carbon dynamics into the soil (Post and Kwon, 2000), has been recognized as an important driver to explain the net carbon flux to the atmosphere (Le Quéré *et al.*, 2015; Houghton and Nassikas, 2017). In fact, soil organic C has been reduced by 10%-59% due to human land-use activities such as the conversion of natural vegetation to agricultural land (Guo and Gifford, 2002), resulting in approximately 35% of historical anthropogenic CO_2 emissions between 1850 and 1990 (Foley *et al.*, 2005). This contributed to global warming and related environmental problems. Currently, because of human disturbances, such as increased tillage frequency in a context of rising demand for food and biofuel from agroecosystems (Schlesinger and Bernhardt, 2013), soil C input may be further reduced, and decomposition processes of SOM accelerated (Lal, 2005).

The main components of SOM are C and N, which then are extremely crucial for determining soil fertility and providing nutrients for plant growth (Troeh and Thompson, 2005). The reduction of soil fertility due to loss of SOM is threatening food security in the context of a rapidly growing human population (Sanchez, 2002). Therefore, one of the key strategies to mitigate climate change and enhance soil fertility, thus sustaining food production, is to increase SOC sequestration in soil (Lal, 2004). The potential of conservation practices to enhance SOC accumulation has to be evaluated by assessing their either short- and long-term effect on soil C dynamics (Rusinamhodzi *et al.*, 2011; Laborde *et al.*, 2019). In fact, different soil C pools may be differently affected by soil management practices, thus being early or late indicators for SOM stock changes. In particular, C pools can be fractionated focusing on its either chemical or physical properties and both type of fractionation aim to separate labile from stable SOM (Branco de Freitas Maia *et al.*, 2013). Chemically fractionated C pools are governed by the resistance of their component to degradation, thus being a momentary picture of a steady state for the soil system. However, recent studies suggest that the rate of degradation may depend more on the accessibility of organic compounds to enzymes or microorganisms (Six *et al.*, 2000a), emphasizing the importance of physical fractionation methods to evaluate long-term effects of soil management on SOM.

1.1.2 The importance of N_2O emissions

N_2O is a potent greenhouse gas, having a global warming potential 273-time greater than that of CO_2 on a 100-year time horizon (Allan *et al.*, 2021). Agricultural activities are the largest source of anthropogenic N_2O emissions (Montzka *et al.*, 2011; Syakila and Kroeze, 2011), which are known to be affected by agroecosystem management practices, including application of N-fertilizers, irrigation system, as well as crop type and residue management (Snyder *et al.*, 2007; Perego *et al.*, 2016; Wagner-Riddle *et al.*, 2017; Lin and Hernandez-Ramirez, 2020).

N_2O is the end product of several biotic and abiotic processes such as nitrifier denitrification (Wrage-Mönnig *et al.*, 2018), co-denitrification (Spott and Florian Stange, 2011), chemodenitrification (Van Cleemput, 1998), dissimilatory nitrate reduction to ammonium nitrification (Skiba and Smith, 1993) and denitrification (Firestone *et al.*, 1980). However, the latter two processes are often considered as the main mechanisms behind N_2O emissions from soil (Parton *et al.*, 1996). Nitrification and denitrification are strongly affected by soil water content and by N availability for microorganisms (Bateman and Baggs, 2005). In particular, water filled pore space (WFPS) is a widely used parameter to predict N_2O emissions: generally, when the soil has >60% WFPS (thus leading to anaerobic conditions), N_2O is generally emitted via denitrification. Conversely, at 35–60% WFPS nitrification is considered the main pathway for N_2O production (Bateman and Baggs, 2005). Besides WFPS, the use of mineral N fertilizers and organic manure in agricultural soils generally increases soil mineral N content, thus enhancing N_2O emissions (Dobbie and Smith, 2003).

Therefore, conventional agriculture practices, such as intensive N fertilization and inefficient irrigation systems, have the potential to increase N_2O emissions by increasing mineral N availability for nitrification/denitrification and by promoting anaerobic conditions into the soil (De Rosa *et al.*, 2016). Since human population is likely to rise up to 8.5 billion in 2030 (and to increase further to 9.7 billion in 2050 and 10.4 billion by 2100) (UN, 2022) and food demand will grow as a consequence, both agricultural land area and N_2O emissions are expected to continue to increase in near future (Mosier and Kroeze, 2000). This means that, the increase in anthropogenic N_2O emissions from the agricultural activities may partially offset the reduction of CO_2 emissions from other sectors, such as the energy supply chain. Therefore, new efficient farming practices need to be introduced to limit soil potential to emit N_2O by increasing N use efficiency of crops.

1.2 Resilient farming practices for climate change mitigation and adaptation

The adoption of sustainable agroecosystems management is key for adapting to climate change while lowering agricultural impact on global warming. Resilient practices can lead to preserve existing C stocks and to remove at the same time C from the atmosphere (Guo and Gifford, 2002), while having a positive impact on food security, agro-industries, water quality and the environment (Tilman *et al.*, 2002). Following Conservation Agriculture (CA) practices is widely indicated as a recommended way to pursue those objectives (FAO, 2011). Besides C sequestration into the soil, also N_2O emissions reduction must be taken into account when planning to develop practices for climate change mitigation (Tian *et al.*, 2020). Using high-efficient fertilization and irrigation systems is a viable strategy to increase resource use efficiency, thus limiting environmental impact of agriculture (Ventrella *et al.*, 2012). In particular micro-irrigation systems, combined with fertigation techniques, are suggested as a measure to reduce N_2O emissions from soils and increase N-use efficiency of crops (Sandhu *et al.*, 2019; Kuang *et al.*, 2021).

1.2.1 Conservation agriculture

Conservation Agriculture (CA) is based on a set of three complementary practices: (i) minimum soil disturbance (i.e., no-tillage and minimum tillage), (ii) crop rotation, (iii) and residue retention/permanent soil cover (through main crop residue retention and/or cover crops [CCs]) (FAO, 2011). In recent years, there has been a growing trend toward CA and in particular toward NT, whose benefits include resource and input savings (Lal, 2008). Several studies report higher SOM content under NT due to more fresh material input and reduced oxygen concentration in the subsoil leading to lower mineralization rates of SOM (Kan *et al.*, 2022). This positive effects of CA are particularly pronounced when applied in combination with residue retention and permanent soil cover practices such as CCs (FAO, 2011; Pittelkow *et al.*, 2015). In fact, the benefits of CCs may include soil C and N accumulation, reduced erosion, weed suppression, and increased crop yield (Schipanski *et al.*, 2014). However, different CCs may play different agro-ecological functions. Gramineous CCs are particularly recommended to enhance soil organic matter accumulation and C sequestration because of producing more biomass residue during and after termination for decomposers (Adetunji *et al.*, 2020), whereas legumes CCs maximize N input because of biological N-fixation, thus offering the opportunity to increase STN and reduce dependence on chemical N-fertilizers (Fiorini *et al.*, 2022). In contrast, intensive tillage operations incorporate biomass residues into the soil, promoting physical contact between fresh organic material and soil microorganisms (Coppens *et al.*, 2006), thus establishing a favourable soil microclimate for residue decomposition (Paustian *et al.*, 1997; Balesdent *et al.*, 2000).

However, the real benefit of no-till for C sequestration, and therefore for climate change mitigation and soil fertility restoration, has been recently questioned (Powlson *et al.*, 2014). In the latter study, the authors argue that the increase of SOC stock in the most superficial layer is not only counteracted by the decrease of it in the deeper layers, but also may be short-term. Literature widely reports accumulation of SOC in the top 10 cm soil layer under NT (West and Post, 2002). Yet, while many studies have found a net increase of SOC stocks under NT (Angers and Eriksen-Hamel, 2008), others have shown no difference or even higher values under CT (Virto *et al.*, 2012), particularly when the whole soil profile is considered (Luo *et al.*, 2010). In fact, Edwards *et al.* (1988) found higher SOC content in plowed soil at 15-20 cm depth than under NT. In a multi-site experiment in eastern Canada, Angers *et al.* (1997) observed that CT may promote SOC accumulation near or at the bottom of the plow layer in comparison with NT.

Soil management practices generally affect residence time of C pools within the soil by regulating soil aggregate dynamics, thus having direct impact on C sequestration and cycling (Tisdall and Oades, 1982; Yagüe *et al.*, 2016). It is well known that that NT leads to better macroaggregate formation and stabilization (Six *et al.*, 2000b), in which SOC is physically and bio-chemically protected (Jastrow, 1996; Six *et al.*, 1998; Bosch-Serra *et al.*, 2017). However, the mechanisms behind SOC stabilization are still not well understood and the real potential of NT to sequester C in the long-term is not completely assessed. To further complicate matters, contrasting information is available on selecting correct species of cover crops for SOC and STN sequestration: in fact, Poeplau and Don (2015) found that both legume and non-legume CCs have similar sequestration potential, whereas other studies suggest that legume CCs (Jian *et al.*, 2020) or grasses CCs (Fageria *et al.*, 2007) may sequester more C and N.

Thus, SOM dynamics and nutrient cycling need to be elucidated and subsequently managed. Recent research has focused on the multiple interactions between soil matrix and biota to exploring the effect of tillage practices on these important dynamic soil properties (Six *et al.*, 2004; Kong *et al.*, 2005). Soil aggregates provide physical protection to soil organic matter (Tisdall and Oades, 1982; Yagüe *et al.*, 2016; Bosch-Serra *et al.*, 2017), and also affect microbial community structure (Hattori, 1988), limit oxygen diffusion (Sexstone *et al.*, 1985), regulate water flow (Prove *et al.*, 1990), determine nutrient adsorption and desorption (Linquist *et al.*, 1997; Wang *et al.*, 2001; Domingo-Olivé *et al.*, 2016), and reduce run-off and erosion (Barthès and Roose, 2002).

All these processes have intense effects on SOM dynamics and nutrient cycling, which in turn affect C sequestration in soils, but also roots development and yield. After assessing the effect of CA practices on C sequestration and on soil fertility restoration, the next step is to improve current irrigation and fertilization techniques to limit N losses and to increase N use efficiency of crops.

1.2.2 Efficient fertilization and irrigation systems

Micro-irrigation systems (i.e., surface and subsurface drip irrigation) are high-efficient irrigation techniques capable to deliver low volume of water near to the root zone of crops using plastic pipes (Wu, 1997). Moreover, with drip irrigation systems, nutrients (such as N) can be supplied directly near the plant roots in multiple applications, ensuring constant nutrient availability during crop growth and sustaining crop yield (Guido *et al.*, 2020). Besides increasing yield potential, splitting N fertilization in multiple applications may, also, prevent N surplus into the soil, thus increasing N use efficiency of crops and limiting N availability for microorganisms (Maris *et al.*, 2015). In fact, increasing N use efficiency and reducing N availability for denitrifiers and nitrifiers is essential for N_2O emissions mitigation (Trost *et al.*, 2013). Evidence of this are many studies reporting lower N_2O emissions under drip irrigation systems than under conventional irrigation techniques (i.e., furrow and sprinkler) (Li *et al.*, 2018; Sandhu *et al.*, 2019; Kuang *et al.*, 2021).

Among drip irrigation systems, subsurface drip irrigation (SDI) can further reduce N losses compared with surface drip irrigation due to an improved spatial N-fertilizer application, and to lower surface soil wetting (Kallenbach *et al.*, 2010; Maris *et al.*, 2015; Wei *et al.*, 2018). In fact, in SDI systems driplines are buried from 15 cm down to 50 cm, depending on soil texture, climate, water source, crop type, and/or tillage depth (Lamm *et al.*, 2006). However, recent studies reported higher N_2O emissions under micro-irrigation systems compared with conventional methods due to more frequent soil drying-wetting cycles, which boosts soil N mineralization rates (Kuang *et al.*, 2018). Thus, further research is needed to fully assess SDI capability in reducing N_2O emissions and increasing N use efficiency of crops.

One of the key aspects that may regulate yield performances and N losses from soil under SDI systems may be dripline spacing. Dripline distance is usually set as an integer multiple of crop row distance and ranges between 70 and 300 cm (Lamm *et al.*, 1997;

Lamm, 2016; Lee *et al.*, 2018). However, reducing dripline distance to match dripline spacing and crop distance may lead to more homogeneous distribution of water and N into the soil, avoiding the formation of soaked and N enriched soil areas (Bosch *et al.*, 1998; Sorensen *et al.*, 2013). Therefore, besides testing environmental and productivity potential in comparison with conventional techniques, also different set-ups of micro irrigation system (for example dripline distance and/or depth) need to be studied to find the optimal characteristics for specific soil and crop types.

1.3 Objectives

The general objective of this thesis is to assess the potential of sustainable, climate-smart, and efficient farming practices for agriculture adaptation to climate change, as well as for global warming mitigation.

The specific objectives are:

(1) to evaluate the yield performances of climate-smart practices (i.e., no-till, cover crops, and subsurface drip irrigation) on maize, soybean, and winter wheat as compared with conventional agriculture practices (moldboard plowing without cover crops and sprinkler irrigation)
(2) to assess the potential of no-till and cover crops to provide biomass, C, and N input to the soil, and how these practices may affect SOC and STN dynamics and accumulation in the short- and long-term.
(3) to measure the effect of contrasting irrigation/fertilization systems (subsurface drip irrigation + fertigation *vs.* sprinkler irrigation + granular application of fertilizers) on N_2O emissions and N use efficiency of maize and soybean.

1.4 Outline of this thesis

The following hypotheses resulted from the objectives of this thesis:

(i) No-till + cover crops may lead to higher biomass input and comparable yield to CT (especially after a transition period) by restoring soil fertility.
(ii) No-till + cover crops may enhance SOC and STN content due to higher C and N input and to increased protection of SOM within soil aggregates.
(iii) SDI + fertigation could be suggested as a viable strategy to sustain crop yield

while reducing environmental impact of agriculture by increasing N use efficiency and lowering N_2O emissions.

The thesis has 3 research chapters, next to a general introduction (Chapter 1) and a general discussion (Chapter 5).

In Chapter 2, we (i) examined how different widespread CCs (i.e. rye; phacelia + white mustard ; italian ryegrass + crimson clover + persian clover; hairy vetch + crimson clover) affect in the short-term yield performance of main crops under NT as compared with no-CCs, during a 3-year crop sequence (i.e. maize, soybean, and maize); (ii) assessed the 3-year effects of CCs treatments on inputs (i.e. biomass, C, and N) to the soil, SOC and C pools concentrations, as well as soil fauna (i.e. microarthropods and earthworms) abundance and diversity.

In Chapter 3, we investigated the long-term effect of no-till (NT) coupled with a grass rather than a legume cover crop (i.e., rye [NT-R] and hairy vetch [NT-V]) on main crop yields, biomass input by cover and main crops, soil aggregation and C and N sequestration rates, in comparison with conventional tillage (CT). The study, lasting nine years, was performed on a winter wheat-maize-soybean crop rotation

In Chapter 4, we evaluated the effect of three irrigation systems (subsurface drip irrigation with a narrow dripline spacing [dripline distance of 70 cm; SDI70] vs. subsurface drip irrigation with a wide dripline spacing [dripline distance of 140 cm; SDI140], vs sprinkler irrigation [SPR]) on yield, N_2O emissions and N-use efficiency of maize and soybean.

Chapter 5 provides a general discussion on main results of the study, and identifies future research needs.

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CHAPTER 2.

Driving crop yield, soil organic C pools, and soil biodiversity with selected winter cover crops under no-till

This chapter is based on:

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Abstract

No-till (NT) and cover crops (CCs) have been repeatedly recommended for building-up resilience of agro-ecosystems, enhancing soil biodiversity, and steering efficient nutrients cycling and yield. Yet, the overall impact of CCs on soil properties and dynamics during transition may highly change depending on CC species and interactions with field condition.

In the present 3-yr field study, we (i) examined how selected CCs (i.e. rye [*Secale cereale* L.]; phacelia [*Phacelia tanacetifolia* Benth.] + white mustard [*Sinapis alba* L.]; Italian ryegrass [*Lolium multiflorum* Lam.] + crimson clover [*Trifolium incarnatum* L.] + Persian clover [*Trifolium resupinatum* L.]; hairy vetch [*Vicia villosa* Roth] + crimson clover) affect yield performance of maize (*Zea mays* L.), soybean (*Glycine max* L. Merr.), and maize under NT, and (ii) assessed the effects of CCs on inputs to the soil (i.e. biomass, carbon [C], and nitrogen [N]), soil organic C (SOC) and pools, as well as microarthropods and earthworms abundance and diversity.

Grain yield during the initial 2-yr period was on average reduced with CCs by 1-23% in maize, and 1-33% in soybean. This effect was less evident with CC residues having low C:N ratio (< 20; i.e. hairy vetch + crimson clover) and erects posture after termination (i.e. rye). Thereafter, CCs had no effect on maize yield the third year.

Soil organic C and pools indicated that (i) the effect of our CC treatments over a 3-yr application is limited to the topmost 5 cm of soil, and (ii) the biomass input with CC residue and its C:N ratio are crucial for boosting soil C cycling. This was also the case for earthworm-related indicators, while arthropods mainly responded to different CCs in terms of evenness. Yet, our results on soil fauna showed that different groups or species need different time for showing effects, thus suggesting that responses may be fully effective in a > 3-yr term.

We concluded that CC mixtures that allow the best compromise between the high amount of residue and the low residue C:N ratio should be preferred for: (i) reducing possible detrimental effects on grain yield of maize and soybean, and (ii) enhancing soil C cycling and biodiversity. Therefore, selecting appropriate CC species in mixtures represents the main challenge at the field level for pursuing both objectives in the shortest

timeframe. Within all options in summer crop sequences, here we reported that mixtures including leguminous cover crops might be primarily considered.

1. Introduction

Sustainable land use and management are essential in the delivery of agro-ecosystem services, including biodiversity conservation, landscape preservation, climate regulation, and food provision (FAO 2019). Various strategies for improving soil quality and nutrient cycling at the field level have been defined by the Environment Directorate General of European Commission (2016). Conservation agriculture practices were reported within these options as effective alternatives to conventional management approaches. Main reasons are positive contribution to (i) building-up resilience of farming systems, (ii) steering efficient nutrients cycling and yield, (iii) enhancing soil biology, and (iv) promoting climate change mitigation and adaptation (Lal, 2015). However, Pittelkow *et al.* (2015) in a global meta-analysis documented a yield reduction for a number of field crops during the transition from conventional tillage (i.e. moldboard plowing plus rotary harrowing) to no-till (NT). These authors showed also that introducing cover crops (CCs) within NT systems should be recommended to limit unfavorable effects during such a transition.

Cover crops indeed increase the rate of biomass input to the soil, thus promoting soil organic matter accumulation (Blanco-Canqui and Ruis, 2020). In addition, CC roots act as “bio-drillers” improving soil structure (Fiorini *et al.*, 2018), and indirectly provide pabulum for the entire biotic community in soil (Menta *et al.*, 2020). Yet, the overall impact of CCs on soil properties and dynamics may highly change depending on CC species within each agroecosystem. Gramineous CCs have the highest potential of biomass production, thus targeting nutrient re-cycling and soil organic matter accumulation (Adetunji *et al.*, 2020; Duval *et al.*, 2016). For instance, the concomitant adoption of NT and rye (*Secale cereale* L.) as CC was shown to sustain yield performance of main crops (Boselli *et al.*, 2020), while enhancing soil quality parameters, and keeping nitrous oxide emissions under control (Fiorini *et al.* 2020a). On the other hand, brassicaceous CCs are widely recognized as highly-effective catch crops and often indicated as the best choice to remediate soil compaction (Blanco-Canqui and Ruis, 2020). Last, leguminous CCs are recognized as the most effective whether maximizing nitrogen (N) input become the priority (Gabriel and Quemada, 2011).

At the field level, CCs are usually cultivated in mixture to pursue more than one agro-ecological function, complementing and synergizing the effects. For instance, leguminous grown together with gramineous generally promote facilitation effects by transferring biologically fixed N, thus increasing biomass production (Rasmussen *et al.*, 2013). Diverging functional plant traits (complementarity) indeed increase niche differentiation (Hooper, 1998) to produce a more complete use of resources (e.g. soil N) (Fridley, 2001). It was previously reported that growing together Italian ryegrass (*Lolium multiflorum* Lam.) and clovers maximizes such a resource use complementarity (Ryan-Salter and Black, 2012). However, while their use as forage crops has been widely studied, there is a lack of knowledge concerning the responses of soil quality parameters and crop yield of the following main crop to Italian ryegrass and clovers cultivation in mixture as CC.

Positive effects due to complementarity of plant traits or facilitation were reported to be boosted also by mixing species belonging to the same botanical family (Elsalahy *et al.*, 2019). This \is the especially the case when these species have contrasting above- and below-ground growing traits. For instance, the cultivation in mixture of hairy vetch (*Vicia villosa* Roth) and clovers may potentially follow this statement and enhance the functional differentiation of above- and below-ground community traits, over space and time. Yet, these hypotheses still need to be corroborated with a multi-year experimental approach.

Other combinations at the field level could be selected with mixtures targeted to different agro-ecological functions, as the case of phacelia (*Phacelia tanacetifolia* Benth.) and white mustard (*Sinapis alba* L.). While earlier studies reported the positive effects of phacelia and white mustard cultivated as CC monocultures, their combined effects on soil quality and crop yield of main crops is still missing. The common trait of these two species in temperate climates is a very fast growth before winter (Brust *et al.*, 2014). In addition, they both are excellent N and P scavengers and their residues have relatively low percentage of lignin and C:N ratio (Justes *et al.*, 2009; Liu *et al.*, 2013; Stivers-Young, 1998), which is pivotal for fast and efficient organic matter humification.

Indeed, differences of biomass inputs (in terms of amount and physico-chemical properties, e.g. C:N ratio) to the soil – as derived by different CC species or mixtures – highly affect the degradation of biomass itself and the inclusion of the deriving fresh organic carbon (C) into soil organic carbon (SOC) through humification (Nicolardot *et al.*, 2001). Concentrations and proportions of SOC fractions between available and recalcitrant C pools are useful indicators of decomposition kinetics and humification degree (Vieira Guimarães *et al.* 2013). The same difference in biomass inputs may have an impact also on soil fauna through modifications in their microhabitat and food resources (Bardgett and Cook, 1998; Menta *et al.*, 2020). Beyond its important role in maintaining soil quality and providing ecosystem services, soil fauna has also been included in soil monitoring programs as bio-indicator (Menta and Remelli, 2020).

Diversity, often using synthetic indices approach (such as Simpson, Pielou and Shannon), and abundance, are the most used parameters applied to soil fauna. Unfortunately, the use of these biological parameters alone can be inadequate to explain soil health and quality exhaustively, since they do not take into account neither the ecological role of each taxon nor alteration in community structure. It is known that some soil fauna groups are particularly sensitive to changes in soil management and may ultimately be informative of soil quality variations (Parisi *et al.*, 2005). However, to select a battery of indicators relevant for specific purposes (such as soil quality assessment), the comparison of different biological descriptors is recommended (Pérès *et al.*, 2011). Some studies have suggested that earthworms can be useful in soil quality assessment in different land uses, due to their key functional role in soil ecosystems and their sensitivity to changes in soil properties and plant cover. According to this feature, earthworm biodiversity, abundance and biomass are also considered useful indicators of soil biological activity and quality (van Eekeren *et al.*, 2009; Kanianska *et al.*, 2016).

The objectives of this study were: (i) to examine how different widespread CCs (i.e. rye [*Secale cereale* L.]; phacelia [*Phacelia tanacetifolia* Benth.] + white mustard [*Sinapis alba* L.]; Italian ryegrass [*Lolium multiflorum* Lam.] + crimson clover [*Trifolium incarnatum* L.] + Persian clover [*Trifolium resupinatum* L.]; hairy vetch [*Vicia villosa* Roth] + crimson clover) affect yield performance of main crops under NT as compared with no-CCs, during a 3-year crop sequence (i.e. maize (*Zea mays* L.), soybean (*Glycine max* L. Merr.), and maize); (ii) to assess the 3-year effects of CCs treatments on inputs (i.e. biomass, C, and N) to the soil, SOC and C pools concentrations, as well as soil fauna (i.e. microarthropods and earthworms) abundance and diversity. The following hypotheses were tested: (i) crop yield is increased by CCs, especially yield of maize by leguminous-based CCs; (ii) CCs treatments with the highest biomass production and lowest biomass C:N ratio are the most effective for enhancing soil quality.

2. Materials and methods

2.1 Field site and treatments

A three-year field study was conducted between September 2016 and October 2019, at the commercial "Ciato farm", located in Panocchia (44°40'20.3"N 10°18'04.5"E; 174 m asl), near Parma, Po Valley, Northern Italy. The soil had a clay loam texture (sand 339, silt 368, and clay 293 g kg^{-1}) in the upper layer (0-30 cm), and was classified as a loamy, mixed, mesic Fluventic Ustochrepts, according to the Soil Taxonomy (NRCS Soil Survey Staff, 2014). Initial soil physical and chemical properties in the 0-30 cm soil layer were: pH 6.5, SOC 10.9 g kg^{-1}, total N 1.1 g kg^{-1}, available P 34 mg kg^{-1}, exchangeable K 131 mg kg^{-1}, and cation exchange capacity 21 cmol^{+} kg^{-1}. The climate is temperate (Cfa as Köppen classification), mean annual temperature is 13.1 °C and annual precipitation is 830 mm.

The field experiment was conducted on a three-year summer-crop sequence with maize (*Zea mays* L.), soybean (*Glycine max* (L.) Merr.), and maize again. Experimental treatments were established in September 2016. Conversion to NT occurred with the experiment starting, since the entire field was previously managed with conventional tillage practices (i.e. moldboard plowing plus rotary harrowing, without cover crops). Cover crops were cultivated from September to middle March in the 2016-2017 winter season, from September to end of March in the 2017-2018 winter season, while from October to middle March in the 2018-2019 winter season. In detail, treatments were: (1) Control, a no-CC treatment as a control; (2) R, a rye monoculture; (3) PM, a two-species mixture composed by phacelia (62%) and white mustard (38%); (4) RCC, a three-species mixture composed by Italian ryegrass (48%), crimson clover (38%), and Persian clover (14%); (5) VC, a two-species mixture composed by hairy vetch (35%) and crimson clover (65%). As a result, the experiment design was a randomized complete block (RCB) with three blocks and five treatments corresponding to the five winter cover crops. Plot size was 2600 m^2 (20 m width and 130 m length). Sowing of CCs took place with a sod-seeder each year, two weeks after having harvested the previous main crop. Seeding rates of CCs were: 100 kg ha^{-1} for R, 25 kg ha^{-1} for PM, 65 kg ha^{-1} for RCC, and 50 kg ha^{-1} for VC. Cover crop termination took place each year right before planting the main crop by spraying 3 L ha^{-1} of Roundup Platinum (Glyphosate 79.5%) in all CC treatments, and in Control treatment to suppress spontaneous weeds. Main crops (i.e. maize and soybean) were planted at a 70-cm row distance. Maize was planted at the beginning of April (in both years); soybean at the beginning of May. Number of plants per square meter was 7.5 and 7.7 for maize (in 2017 and 2019, respectively), and 38.5 for soybean. Both maize and soybean were irrigated by traveling sprinkler. Fertilizations occurred for maize (220 kg N ha^{-1} as urea), with two applications (100 kg N ha^{-1} at V2-3 and 120 kg N ha^{-1} at V6-7), at the same rate for all treatments. Harvest took place at the beginning of September for Maize (in both years), and at the end of September for Soybean.

2.2 Plant biomass sampling and analyses

Total aboveground biomass of the main crops and CCs was measured every year right before harvest and termination, respectively. Biomass samples were collected from three random areas of 6 m^2 within each plot for main crops, and of 4 m^2 for CCs. In the case of main crops, grain was manually separated from the crop residue. Right after, the main crop was harvested by combine and all the grain from each plot was weighted and sampled separately.

Once in the lab, grain samples were dried at 105 °C for 24 hours and weighted to determine crop yield. Residue samples (of the main crops and CCs) were dried at 65 °C until constant weight and then ground at 1 mm size. Then C and N concentrations were determined for all residue samples by the Dumas combustion method with an elemental analyzer (Vario Max CNS, Elementar, Germany). Residue-derived C and N inputs to the soil for each crop (main crop and cover crop) was calculated by multiplying the weight of biomass by their C and N concentrations. 3-yr cumulative biomass, C, and N, input with main crop and cover crop residue separately were calculated. 3-yr average C:N residue of both main crop and cover crops was also computed.

2.3 Soil sampling and analyses

Soil sampling took place at the end of the experiment (October 2019), immediately after harvesting maize. Within each plot, six soil sub-samples at 0-30 cm soil depth were collected using a coring device with a 15-mm diameter auger. After extraction, each soil core was divided into three portions according to the three different soil layers: 0-5 cm, 5-15 cm, and 15-30 cm. The six sub-samples of each layer for each plot were pooled together and mixed. As a result, the total number of soil samples was 45. Samples were then air dried, passed at 2-mm sieve and analyzed. Soil organic carbon (SOC) concentration was determined as Walkley & Black method (Nelson and Sommers, 1996). Total extractable carbon (TEC), and humic and fulvic acid carbon (HA + FA) were determined according to Nelson and Sommers (1996) with the dichromate oxidation method. Not humified and more labile C fraction (NHC) was calculated as follows:

$$\mathrm{NHC} = \mathrm{TEC} - (\mathrm{HA} + \mathrm{FA}).$$

Not extractable organic carbon (NEC), conventionally defined as humin (a pool of organic carbon recalcitrant to microbial degradation), was calculated as the difference between SOC and TEC (2):

$$\mathrm{NEC} = (\mathrm{SOC} - \mathrm{TEC}).$$

Humification rate (HR) was determined according to Francaviglia *et al.* (2017) as follows:

$$\mathrm{HR} = (\mathrm{HA} + \mathrm{FA} \times 100) / \mathrm{SOC}.$$

2.4 Microarthropod-based soil quality evaluation

For soil arthropod extraction, within each plot, three soil cubes of 10×10×10 cm were collected using a spade after removing the superficial litter. Also in this case, soil sampling took place at the end of the experiment (October 2019), immediately after maize harvesting. The soil samples were carried to the lab within 24 hours. Arthropods were extracted using the Berlese-Tullgren funnel (2 mm mesh size, extraction time 10 days) and preserved in a 70% ethanol and 30% glycerol solution. The extracted specimens were identified at class level for Myriapoda and order level for Crustacea, Hexapoda and Arachnida using a stereomicroscope (20-40×). All the specimens belonging to each taxon were counted to obtain abundance data (expressed in individuals m^{-2}). For each plot, Simpson Index of diversity (1-D), Shannon Diversity Index (H) and Pielou's evenness (J) were applied to arthropod data.

To evaluate the microarthropod-based soil quality, QBS-ar index was applied (Parisi *et al.*, 2005). This index is based on the positive relation between the number of arthropod groups adapted to soil and the soil biological quality. Indeed, soil arthropods show morphological characters revealing their adaptation to soil habitat. Higher morphological adaptation to soil indicates higher sensitivity to chemical and physical variation, and, consequently, to soil degradation. Therefore, a higher soil quality would be related to a higher number of well adapted microarthropod groups. QBS-ar index is based on the morphological characters mentioned above, assigning at each taxon, an Eco-Morphological index (EMI), ranging between 1 and 20, in relation to the adaptation level to soil (1 = no adaptation; 20 = best adaptation). QBS-ar results from the sum of each maximum EMI score assigned at each taxon identified in the soil sample. For more details, see Menta *et al.* (2018).

2.5 Earthworm sampling and counting

Three undisturbed soil cubes of 8,000 cm^3 (20×20×20 cm) were collected from each plot by a spade and brought to the lab within 24 h. Thereafter, earthworms were manually separated from the soil and counted to determine the number of individuals (Shepherd *et al.*, 2008). Before being weighted, earthworm intestines were voided according to (Dalby *et al.*, 1996). Thus, the earthworm density (number of earthworms per square meter) and the earthworm biomass (g of earthworms as dry biomass per square meter) were calculated by multiplying the number and the dry biomass of earthworms extracted from each undisturbed soil cubes by 25.

2.6 Statistical analyses

Data on (i) grain yield of main crops (i.e. maize 2017, soybean 2018, and maize 2019), (ii) 3-yr inputs (i.e. biomass, C, and N) to the soil due to main crop and CC residues, (iii) SOC and C pools concentrations (i.e. TEC, HA+FA, NHC, and NEC), as well as (iv) humification index (i.e. HR), were statistically analyzed with linear analyses of variance (ANOVA) by using the "agricolae" package of RStudio 3.3.3 (R Core Team, 2020). Similarly, ANOVA was applied to test for differences between treatments on both microarthropod and earthworm data. The variables considered were arthropod total abundance, abundance of arthropods showing EMI 20, number of eco-morphological groups, and number of eco-morphological groups with EMI 20, the indexes (i.e. Simpson, Shannon, Pieolu's evenness and QBS-ar), earthworm's density and weight. Tukey test was performed as *post-hoc* in within treatments comparisons; while Dunnett test, using R package "DescTools" (Signorell *et al.*, 2020), was used to compare treatments with Control. Models for multiple linear regression were carried out with microarthropod and earthworm variables and biomass input and C:N ratio (of main crop + CC residues) as terms.

All variables were examined for normality with Shapiro-Wilk test and for homogeneity of variances with Levene's test prior to perform the analyses. Mean values were separated with Tukey's test ($\alpha = 0.05$) by using the "multcomp" package. Only TotEMG data, which did not show a normal distribution, were square root transformed.

Arthropod community matrix was square root transformed to minimize the influence of the most abundant groups and Bray-Curtis dissimilarity index was calculated. Then, permutational multivariate analysis of variance (PERMANOVA) was conducted on the dissimilarity matrix, considering treatments as independent variables, and using the R package "RVAideMemoire*"* for pairwise comparisons. Data were visualized with non-metric multidimensional scaling (NMDS) and hierarchical clustering. An analysis of similarity percentages (SIMPER) was then performed to test which arthropod groups were driving the differences in assemblages. Ordination, PERMANOVA and SIMPER were all performed using the R package "vegan*"* (Oksanen *et al.*, 2008).

3. Results

3.1 Grain yield

Grain yield was significantly affected by CC treatment in maize 2017 (Figure 1a) and in soybean 2018 (Figure 1b), while not in maize 2019 (Figure 1c). In detail, maize grain yield in 2017 was the highest under Control and R, and progressively decreased in the order VC ≥ PM ≥ RCC.

Control had the highest grain yield also with soybean in 2018 (together with VC in this case), followed by RCC, PM, and R (Figure 1).

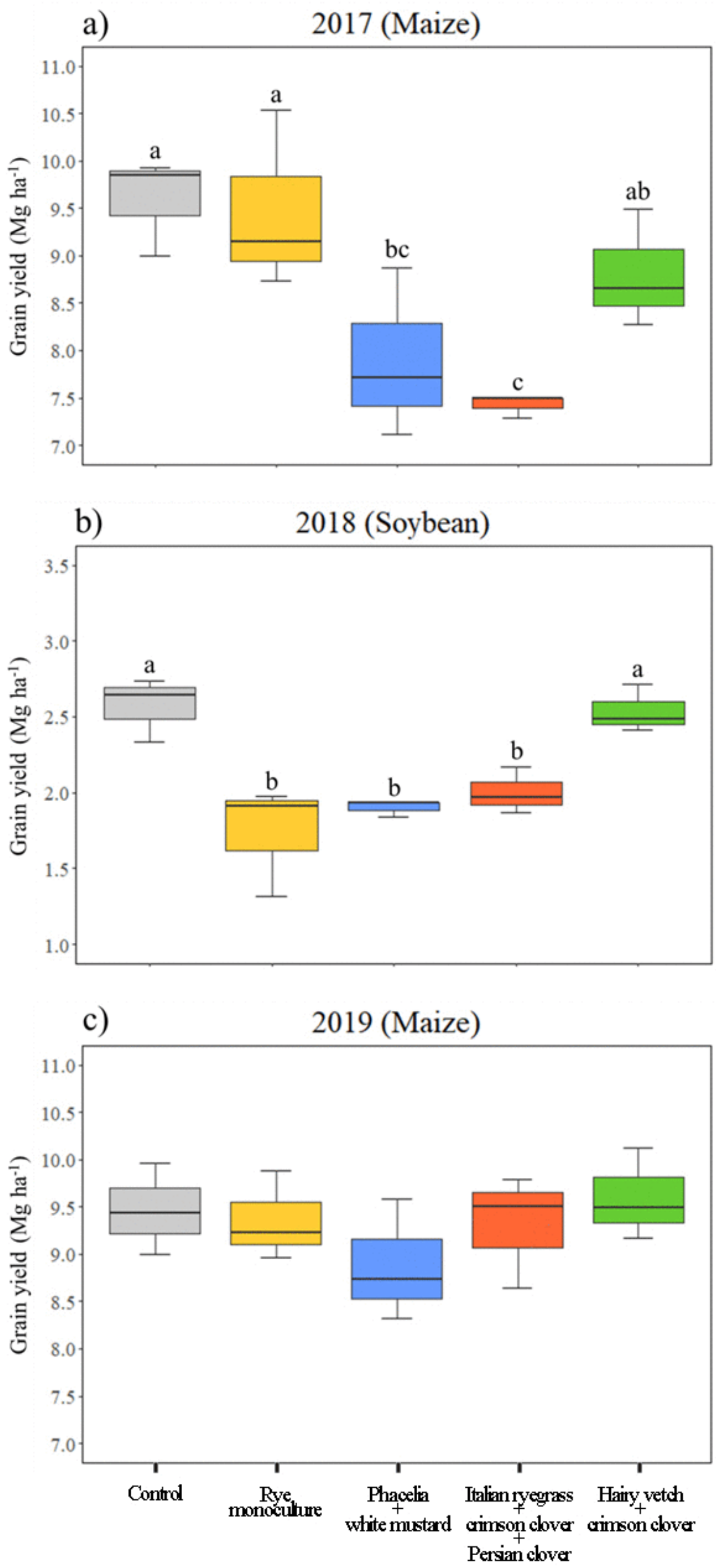

Figure 1. Grain yield (Mg ha^{-1}) of maize (a), soybean (b), and maize (a) during the 3-yr field study as affected by treatment: control (Control); rye (R); phacelia + white mustard (PM); Italian ryegrass + crimson clover + Persian clover (RCC); hairy vetch + crimson clover (VC). Different letters above bars mean significant differences between treatments.

3.2 Residue-derived biomass, C, and N inputs

The 3-yr cumulative biomass and C inputs to the soil due to main crops residue were not affected by CC treatment, although values tended (p between 0.06 and 0.08) to be higher under Control, R, and VC, and lower under RCC and PM (Table 1). The 3-yr cumulative N input due to main crop residue was significantly higher under Control and VC than under RCC, R, and PM. No difference was found in 3-yr average C:N ratio.

The cumulative 3-yr biomass input to the soil due to CC residue (which excluded Control by definition) was significantly lower under VC than under the other CC treatments (Table 1). The cumulative 3-yr C input was not statistically affected. Nevertheless, VC tended ($p = 0.0522$) to have the lowest 3-yr cumulative values also in this case. Conversely, the cumulative 3-yr N input due to CC residue was significantly higher under VC than under RCC, R, and PM. This turned into the lowest C:N ratio for VC residues.

3.3 Soil organic C and pools

3-year CC treatments significantly affected SOC concentration in the 0-5 cm soil layer (Table 2): PM had the highest SOC concentration, Control was the lowest, while all the others CC treatments were not different from both the former and the latter. Such a significant effect of CC treatment in SOC concentration was not recorded in the 5-15 cm and in the 15-30 cm soil layer (Table 2). Nevertheless, differences among treatments were close to be significant (at least in the 5-15 cm, with a p-value of 0.0875), but the treatments hierarchy did not follow the same pattern as in the 0-5 cm soil layer. In detail, R and VC treatments tended to increase SOC concentration in the 5-15 cm and 15-30 cm soil layer, respectively; RCC and Control had always the lowest SOC concentration values; PM led to an intermediate value of SOC concentration in both soil layers.

Residue	Treatment	3-yr cumulative biomass input (Mg ha^{-1})	3-yr cumulative C input (Mg ha^{-1})	3-yr cumulative N input (kg ha^{-1})	3-yr average C:N ratio
Main crops residue	**Control**	35.60	16.84	259.61 a	62
	R	33.77	16.29	218.28 b	75
	PM	29.51	14.67	220.36 b	67
	RCC	27.42	13.46	212.63 b	64
	VC	33.29	16.45	255.00 a	65
	p (F)	0.0675	0.0740	0.0288	0.1235
CCs residue	**R**	7.42 a	3.68	157.59 b	24 c
	PM	6.61 b	2.90	150.16 b	19 b
	RCC	7.65 a	3.62	157.59 b	24 c
	VC	4.18 c	2.00	193.92 a	10 a
	p (F)	0.0125	0.0522	0.0426	0.0034

Table 1. 3-yr cumulative biomass, C (Mg ha^{-1}), and N input (kg ha^{-1}), as well as average C:N ratio, as affected by treatments, in main crop (upper part) and cover crop (lower part) residue. Control; R: Rye; PM: Phacelia and white Mustard; RCC: italian Ryegrass, crimson Clover, and persian Clover; VC: hairy Vetch and crimson Clover. Lowercase letters indicate differences among treatments within the same type of residue. P-values by ANOVA are also reported.

Total extractable carbon (TEC) and HA+FA concentrations in the 0-5 cm soil layer were significantly affected by CC treatment (Table 2). Both R and PM had the highest concentrations in this case, then RCC and Control had the lowest TEC and HA+FA, while VC showed intermediate values. As regard the 5-15 cm and the 15-30 cm soil layers, TEC and HA+FA concentrations were not statistically affected by treatment (Table 2). Nevertheless, concentration values were always the lowest for both the two fractions and two soil layers under C.

Similarly to SOC concentration, NEC was found to have the highest concentration under PM (and also under RCC in this case) in the 0-5 cm soil layer, while under R (although without statistical significance; p-value 0.0799) in the 5-15 cm soil layer (Table 2).

The HR showed significant differences among CC treatments in the 0-5 cm soil layer, while not in the deeper ones. In detail, R had the highest HR, Control and RCC the lowest, while PM and VC showed intermediate values (Table 2)

Soil depth	Treatment	SOC	TEC	HA+FA	NHC	NEC	HR
		Pool amount (g kg^{-1} soil)					(Humification)
0-5 cm	**Control**	12.19 c	1.99 b	1.11 b	0.89	10.50 b	8.88 b
	R	13.17 b	2.32 a	1.32 a	1.00	10.85 ab	10.04 a
	PM	13.70 a	2.28 a	1.28 a	1.02	11.22 a	9.31 ab
	RCC	13.15 b	2.00 b	1.10 b	0.91	11.17 a	8.34 b
	VC	12.99 b	2.21 ab	1.19 ab	1.02	10.54 b	9.17 ab
	p (F)	0.0337	0.0427	0.0458	0.5887	0.0403	0.0478
5-15 cm	**Control**	11.06	1.73	1.03	0.71	9.33	9.29
	R	12.11	1.82	1.12	0.71	10.28	9.23
	PM	11.68	1.86	1.18	0.68	9.82	10.09
	RCC	11.16	1.87	1.12	0.74	9.29	10.04
	VC	11.76	1.80	1.10	0.71	9.95	9.33
	p (F)	0.0875	0.4063	0.3073	0.9812	0.0799	0.2696
15-30 cm	**Control**	10.59	1.62	0.92	0.70	8.96	8.74
	R	11.32	1.82	1.08	0.74	9.50	9.53
	PM	10.77	1.76	1.04	0.72	9.01	9.68
	RCC	10.40	1.71	0.98	0.73	8.69	9.41
	VC	11.43	1.82	1.02	0.80	9.61	8.91
	p (F)	0.1282	0.1080	0.5171	0.7828	0.2017	0.7521

Table 2. Concentration (g kg^{-1} soil) of soil organic carbon (SOC), total extractable carbon (TEC), humic and fulvic acid carbon (HA+FA), not humified carbon (NHC), and not extractable organic carbon (NEC), as well as humification rate (HR), in different soil layers (0-5 cm; 5-15 cm; 15-30 cm) as affected by 3-yr cover crop treatment. Control; R: Rye; PM: Phacelia and white Mustard; RCC: italian Ryegrass, crimson Clover, and persian Clover; VC: hairy Vetch and crimson Clover. Lowercase letters indicate differences among treatments within the same type of residue. P-values by ANOVA are also reported.

3.4 Soil arthropods

Our results on the abundance of eco-morphological groups showed that a minimum of 4 and a maximum of 9 groups were identified, for a total number of arthropods ranging between 191 ind. m^{-2} and 552 ind. m^{-2} (Table 3). Among all arthropods extracted, 43% were Acari, 29% Collembola, 7% Coleoptera (adults:larvae in a ratio of 1:1), 7% Psocoptera, 6% Hymenoptera, 3% Diplura and 1% Chilopoda. Araneidae, Isopoda, Symphyla, Hemiptera and others Holometabola account each one for less than 1%. No significant difference was found in total abundance and in abundance of microarthropods with EMI 20, and neither in the total number of eco-morphological groups while the number of groups having EMI 20 showed a significant increase under RCC compared to the Control ($p < 0.05$) (Table 3). Such a difference was mainly due to the presence of Collembola with EMI 20 and Chilopoda.

In the present study, neither Simpson index (1-D) nor Shannon index (H) differed significantly within treatments and between them and the Control, while Pielou's evenness (J) differed only within treatments ($p < 0.05$; Figure 2a, b, and c, respectively). Nevertheless, PM always showed the lowest value, while R and VC constantly highlighted the highest ones (although significantly only with Pielou's). Last but not least, the QBS-ar index was also not significantly affected by treatment (Figure 2d) in our experiment, although Control tended to have the lowest value.

No arthropod-based variable resulted explained by multiple linear regression models with C:N ratio and biomass input terms (data not shown).

PERMANOVA analysis showed that arthropod assemblages differed between treatments ($p < 0.01$; Figure 3a); however, no pairwise comparison resulted significant. SIMPER analysis showed that treatment communities differed one to each other for less than 50%, with major dissimilarities in R *vs* PM and R *vs* RCC. Those differences were driven in both cases by Psocoptera and Coleoptera larvae, with the addition of Acari in the first contrast and Collembola in the second one (Table A1). Overall, Coleoptera was one of the most important taxa for discriminating between treatments. From the community structure analysis, three clusters emerged: R on one side, PM and RCC on the other, and VC and Control in the middle, thus supporting the NMDS representation (Figure 3b). In this background, Hymenoptera, despite their lower abundance, was the group that more often influence treatments community dissimilarities.

	Control	R	PM	RCC	VC
Acari	155.69 ± 37.45	162.76 ± 69.70	169.84 ± 0.00	134.46 ± 7.08	127.38 ± 32.43
Araneidae	-	7.08 ± 7.08	7.08 ± 7.08	-	-
Isopoda	-	14.15 ± 7.08	-	-	-
Chilopoda	-	-	7.08 ± 7.08	7.08 ± 7.08	7.08 ± 7.08
Symphyla	-	7.08 ± 7.08	7.08 ± 7.08	-	-
Coleoptera	14.15 ± 14.15	14.15 ± 14.15	-	-	35.38 ± 7.08
Collembola	99.07 ± 35.38	56.61 ± 7.08	70.77 ± 7.08	134.46 ± 35.38	70.77 ± 14.15
with EMI 20	7.08 ± 7.08	7.08 ± 7.08	-	21.23 ± 0.00	35.38 ± 18.72
Diplura	7.08 ± 7.08	7.08 ± 7.08	7.08 ± 7.08	21.23 ± 0.00	7.08 ± 7.08
Hemiptera	-	-	-	-	7.08 ± 7.08
Hymenoptera	21.23 ± 0.00	14.15 ± 14.15	7.08 ± 7.08	14.15 ± 14.15	42.46 ± 0.00
Psocoptera	21.23 ± 0.00	63.69 ± 32.43	-	14.15 ± 7.08	28.31 ± 18.72
Others Holometabola	-	7.08 ± 7.08	-	7.08 ± 7.08	-
Coleoptera (larvae)	-	21.23 ± 12.26	-	42.46 ± 42.46	-
Total abundance	325.53 ± 67.51	382.14 ± 139.21	275.99 ± 42.46	396.29 ± 78.80	360.91 ± 63.69
of which with EMI 20	14.15 ± 7.08	21.23 ± 12.26	21.23 ± 21.23	49.54 ± 7.08	49.54 ± 18.72
n° of eco-morphological groups	4.67 ± 0.67	6.33 ± 1.33	6.00 ± 0.58	5.33 ± 0.88	5.67 ± 0.66
of which with EMI 20	1.33 ± 0.33^{b}	2.00 ± 0.58^{ab}	3.00 ± 0.58^{ab}	3.33 ± 0.33^{a}	2.33 ± 0.33^{ab}

Table 3. Abundance of eco-morphological groups (ind. m^{-2}), total arthropods abundance (ind. m^{-2}), abundance of arthropods with EMI 20 (ind. m^{-2}), total number of eco-morphological groups and number of eco-morphological groups with EMI 20, as affected by 3-yr cover crop treatment. Control; R: Rye; PM: Phacelia and white Mustard; RCC: italian Ryegrass, crimson Clover, and persian Clover; VC: hairy Vetch and crimson Clover. Mean values ± Standard Error. Different superscript letters in the variables used for statistical analysis mean significant differences between treatments.

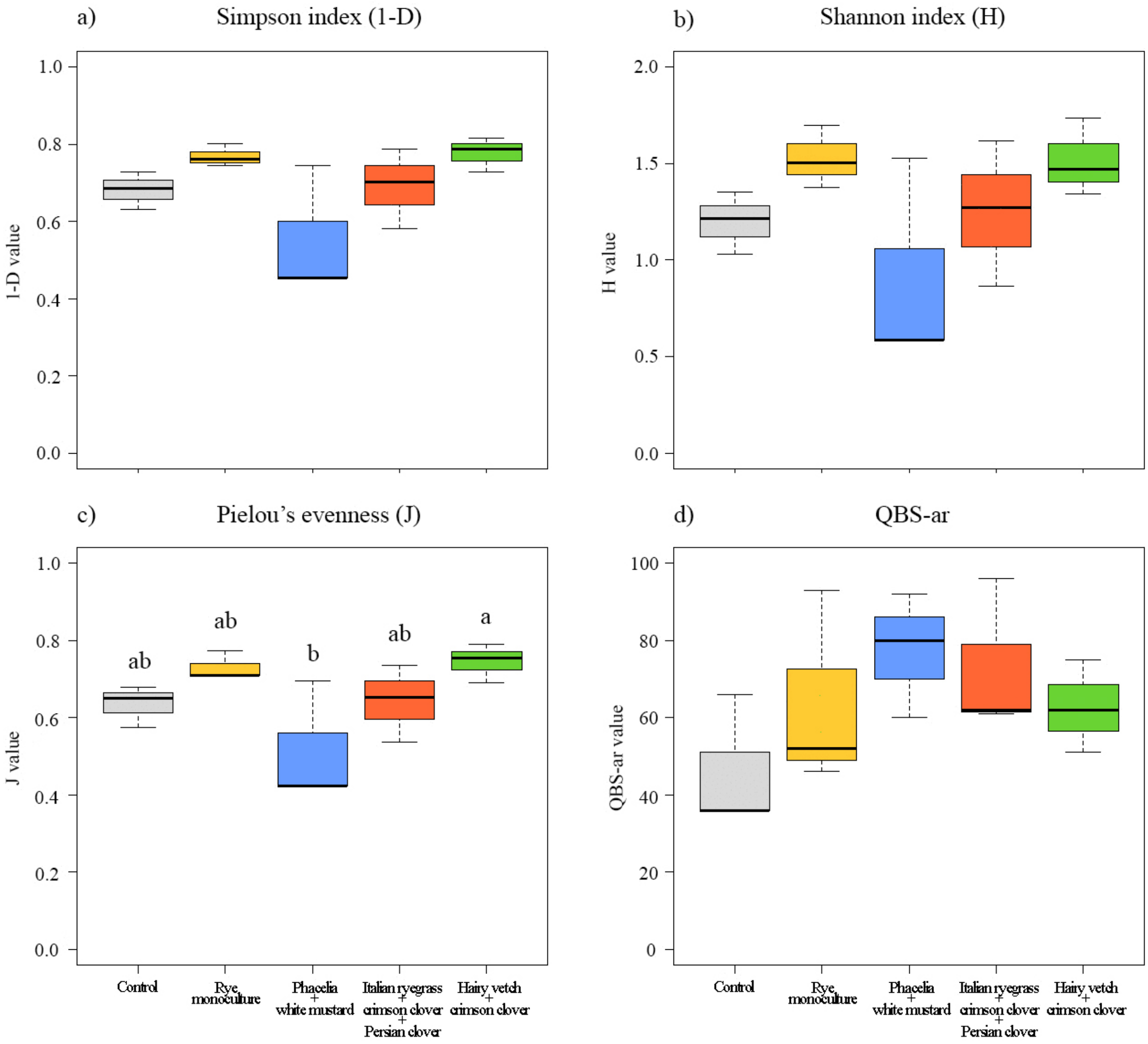

Figure 2. Box-plots of (a) Simpson index, (b) Shannon index, (c) Pielou's evenness, and (d) QBS-ar index for each treatment. The bottom and top of each box represent the lower and upper quartiles respectively, the line inside each box shows the median and whiskers indicate minimal and maximum observations. Different letters above bars mean significant differences between treatments: control (Control); rye (R); phacelia + white mustard (PM); Italian ryegrass + crimson clover + Persian clover (RCC); hairy vetch + crimson clover (VC).

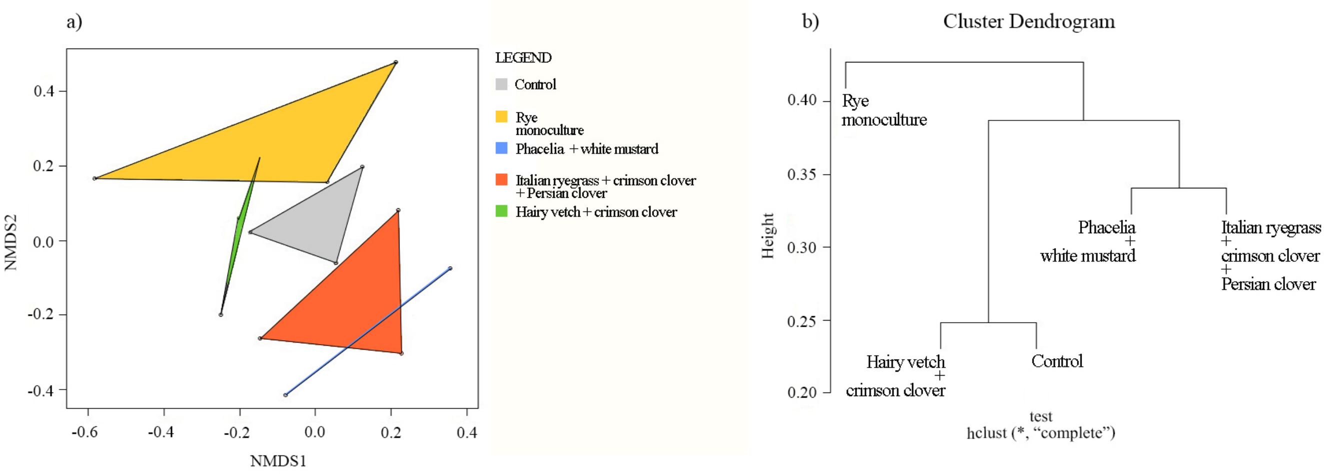

Figure 3. (a) NMDS ordination plot (stress: 0.1636321), and (b) hierarchical clustering on Bray-Curtis dissimilarities in arthropods communities according to treatments: control (Control); rye (R); phacelia + white mustard (PM); Italian ryegrass + crimson clover + Persian clover (RCC); hairy vetch + crimson clover (VC). Different letters above bars mean significant differences between treatments.

3.5 Earthworms

The treatment RCC had the highest value of earthworm abundance, followed by VC, while Control was the lowest. Both RCC and VC abundance significantly differed from the Control (Figure 4a). Earthworm weight showed a similar pattern, with the highest value in RCC and VC and the lowest in Control. (Figure 4b). Differences were observed between treatments ($p \leq 0.01$), with RCC and VC significantly higher when compared to R, while no differences were observed when compared with PM. Conversely, both earthworm abundance and weight were explained (for at least 50% of their variance and with a $p<0.01$) by a multiple regression model using C:N ratio and biomass input terms, in the present study. Nevertheless, the C:N ratio was the only one affecting (negatively) the dependent variables, both for abundance and weight model ($p<0.01$ and $p<0.001$, respectively).

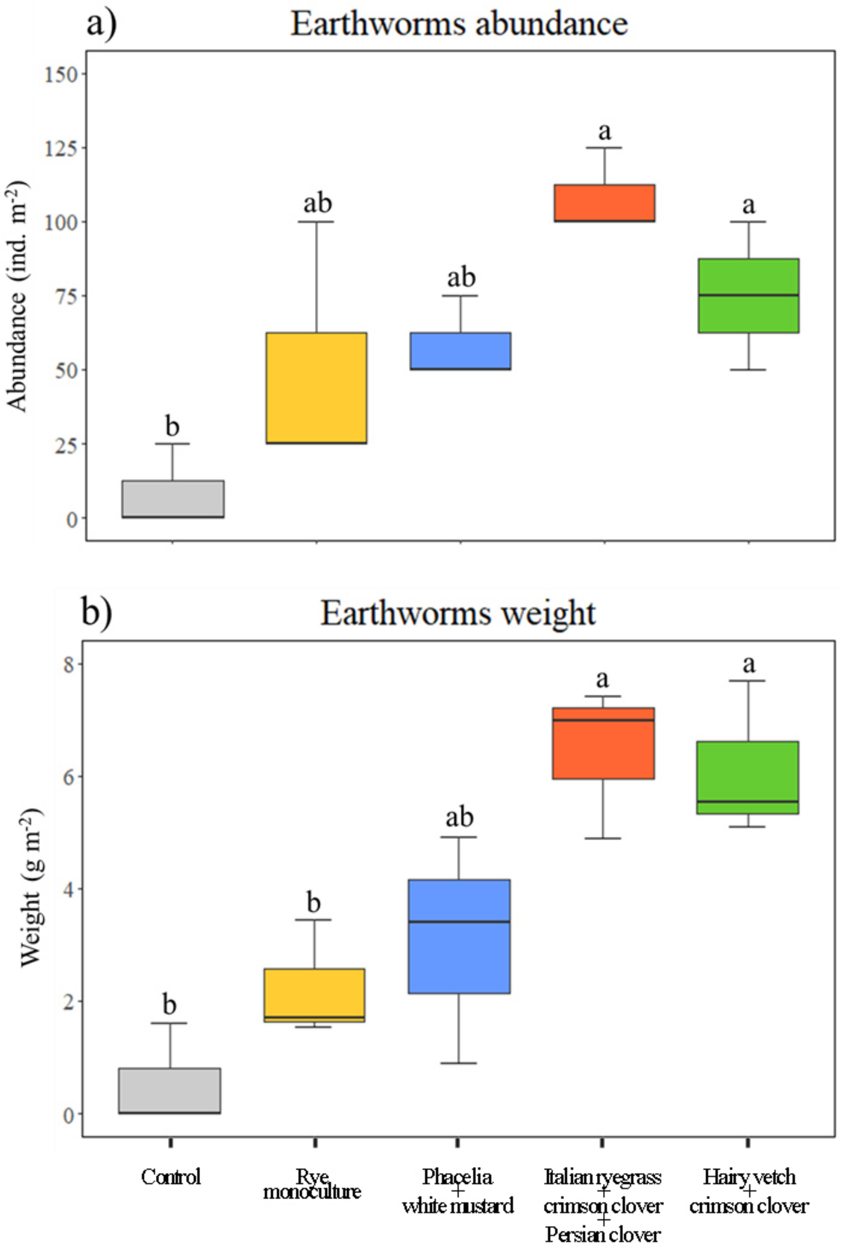

Figure 4. Box-plots of (a) earthworm abundance and (b) earthworm weight for each treatment. The bottom and top of each box represent the lower and upper quartiles respectively, the line inside each box shows the median and whiskers indicate minimal and maximum observations. Different letters above bars mean significant differences between treatments: control (Control); rye (R); phacelia + white mustard (PM); Italian ryegrass + crimson clover + Persian clover (RCC); hairy vetch + crimson clover (VC).

4. Discussion

4.1 Effect of cover crops on grain yield of maize and soybean

The highest maize yield under Control in the present study is in apparent contradiction with previous outcomes reporting that negative effects of NT on crop yield in the initial years might disappear in the case of concomitant inclusion of CCs (Boselli *et al.*, 2020; Pittelkow *et al.*, 2015). Main reasons reported for such a positive effect of CCs were: the increased soil organic matter and nutrient cycling due to extra-inputs of biomass (Blanco-Canqui *et al.*, 2011), as well as the "bio-drilling" function of CC roots improving soil structure (Fiorini *et al.*, 2018). However, other authors (Calonego and Rosolem, 2010) showed that soil compaction during transition to NT remains a main issue in the very initial years in spite of the concomitant inclusion of CCs, since their actions are gradual, being fully effective in a 3- to 4-year term. Our results are in agreement with this second statement and showed no positive yield effects of CCs in the very short term (2017 and 2018).

In addition, the highest grain yield under Control also with soybean in 2018 (together with VC in this case) suggests that yield responses to CC treatment in our experiment were associated to other than factors related to differential soil compaction and/or root development. A possible explanation is that CC residue may have had negative effects on the initial phenological phases of main crops. It is well known indeed that NT *per se* reduces soil temperature and delays emergence and initial rooting of crops planted in early spring under temperate climate (Wang *et al.*, 2012). Then, combining certain (i.e. PM and RCC on maize in 2017, R, PM, and RCC on soybean in 2018) CCs and NT may have further boosted this effect, thus leading to a reduced yield under certain CC treatments compared with under Control in our study. Similar results were previously reported by Salmerón *et al.* (2011) under similar soil-climate conditions.

Yet, certain other CCs (i.e. VC and R on maize in 2017, VC on soybean in 2018) had no effect on crop yield in our experiment. This was probably because of novel aspects not considered before: on one side, beyond the highest related N input, VC residue – with a low C:N ratio – underwent to a fast decomposition in both years, thus limiting the effect of reducing soil temperature; on the other side, R residue might have behaved as VC residue with respect to soil temperature, but mainly because of its erect posture also after termination, and only in the case of early termination timing (middle of March as in 2017) with a relatively low residue amount.

4.2 Responses of residue-derived biomass, C, and N input

Overall, Control in our study increased (or tended to increase) biomass, C, and N input due to main crops residue compared with CC treatments probably because of a differential growth performance as a consequence of presence/absence of CC residues (as discussed above). Previous findings indeed suggested that a delayed plant growth during the initial stages often results into a reduced plant height, which negatively affect the amount of biomass, C, and N input to the soil as crop residue (Dam *et al.*, 2005).

On the other hand, our results on CC residue showed that the gramineous-based CCs had the highest values of cumulative 3-yr biomass and C input, while VC had the lowest ones. Conversely, VC the highest cumulative 3-yr N input, which turned into the lowest C:N ratio for VC residues. Taking into account climate variability, these results confirmed that gramineous-based CCs are generally those with the highest productivity potential (both in term of biomass and C) under NT (Duval *et al.*, 2016). Yet, whether maximizing N input become the priority legumes will be more effective (Gabriel and Quemada, 2011).

4.3 Impact of cover crop on soil organic C and pools

Our results showed that SOC concentration in the 0-5 cm soil layer was increased by all the tested 3-year cover crops, being PM the CC treatment leading to the highest SOC increase. Conversely, no significant effect was recorded in the 5-15 cm and in the 15-30 cm soil layer, although R and VC tended to have the highest SOC concentration in the 5-15 cm and 15-30 cm soil layer, respectively. RCC and Control had always the lowest SOC concentration values; PM led to an intermediate value of SOC concentration in both soil layers. These results highlight that the tested CCs have the potential to boost SOC accumulation, even though not at the same extent. Additional biomass (and C) input due to CCs cultivation may indeed increase SOC concentration due to the extra-amount of crop residues (Duval *et al.*, 2016). However, such an effect is often limited to the topmost centimeters of soil if NT is adopted and direct inputs to the deeper soil by plowing are suspended (Boselli *et al.*, 2020).

A 3-year period of PM cultivation as winter CC was the best option to increase SOC concentration in the 0-5 cm soil layer in the tested soil-climate condition. PM was better than gramineous-based CCs (i.e. R and RCC), although R and RCC had higher 3-yr cumulative biomass input (and tended to have also higher 3-yr cumulative C input) to the soil than PM. This suggests that other than biomass and C inputs were the main drivers regulating SOC concentration, at least in the short term prospective. In particular, the C:N ratio of PM residues was lower than that of R and RCC, which may have promoted the degradation of residues and the inclusion of the deriving C into the SOC through humification (Nicolardot *et al.*, 2001). Higher humification coefficients with lower C:N residue are widely recognized (Hättenschwiler and Gasser, 2005; Nicolardot *et al.*, 2001). However, this was not the case of VC treatment although the lowest C:N ratio of residues and the highest humification rate, because of the much lower biomass input (between – 37% to – 65%) than all the other CC treatments

As regards soil C pools, TEC and HA+FA concentrations in the 0-5 cm soil layer were observed to be increased by R in the present study. This was probably due to (i) the relatively high rhizodeposition reported for rye in earlier studies (Austin *et al.*, 2017), and (ii) the increased 3-yr biomass inputs to the soil with main crops and CC residue under R (41.19 Mg ha^{-1}), which together stimulated TEC and HA+FA accumulation processes (Francaviglia *et al.*, 2017). While for PM, the low C:N ratio of CC residue may explain TEC and HA+FA concentrations since it is indicative of a fast decomposition rate and a high humification degree (Vieira Guimarães *et al.* 2013). Then, no difference was recorded in the 5-15 cm and 15-30 cm soil layers. Nevertheless, we found that Control had the lowest values for both TEC and HA+FA concentrations in both layers. This could be ascribed to the lower abundance and activity of soil fauna (i.e. earthworms) under Control (as reported above), which may have reduced the incorporation of organic matter down to deeper soil layers (Pulleman *et al.*, 2005).

As for SOC concentration, also NEC was found to have the highest values under PM in the 0-5 cm soil layer, while under R in the 5-15 cm soil layer. According to Camilli *et al.* (2016), higher concentration of NEC indicates the presence of a C pool less sensitive to mineralization and stabilized in chemically or physically protected stable forms. Results presented here corroborates this previous finding and a very close relationships between NEC and SOC concentration.

Last, also HR showed significant differences only in the 0-5 cm soil layer and the ranking was R ≥ PM = VC ≥ Control = RCC. Since HR refers to the humified C fraction compared to SOC, a high HR ratio is generally indicative of a low degree of humification (McCallister and Chien, 2000). The high HR observed under R is assumed to be related to the greater content of non-humic substances and non-decomposed material, thus corroborating the slow decomposition rate of the residues left onto the soil surface under this CC treatment.

4.4 Effects on soil fauna

The most popular parameters observed to characterize soil invertebrate communities are diversity and abundance (Menta & Remelli, 2020). In this study, no evidences of cover crop impact on those parameters were highlighted, a result that is in apparent agreement with findings of Menta *et al.* (2020), who reported that those variables are often affected more by main crop type or sequence, rather than by residue management or cover cropping. Our results also suggest that leguminous-based CCs (as RCC and VC in our study) may have some positive effects in a longer period of time, at least on the abundance of arthropods which are more adapted to soil (EMI 20), and consequently more sensitive to soil conditions. Highly-adapted Collembola and Chilopoda may have taken advantage especially from RCC conditions, in accordance with Salamon *et al.* (2004), who found that the identity of plant species in a mixture is an important determinant for springtails, especially if legumes are involved. Indeed, they argued that legumes increase Collembola diversity through increasing microbial (particularly fungal) biomass in the rhizosphere. Furthermore, the N-rich litter of legumes forms an attractive food resource for both Collembola and Chilopoda (Menta and Remelli, 2020). Previous results by Fernández *et al.* (2008), who studied the contribution of CCs in the development of sustainable agriculture scenarios, found that legumes constantly hosted the highest arthropods biodiversity. In this study, biodiversity indexes results suggested that PM gave the worst effects on soil fauna, however from QBS-ar emerged that this cover crop hosted arthropods better adapted to soil; only legumes maintain overall high values for all the parameters considered. Nevertheless, QBS-ar results substantially agrees with previous studies (Sapkota *et al.* 2012; Fiorini *et al.* 2020b) suggesting that (i) biomass *vs* no biomass input could be considered as a main driver of QBS-ar pattern, and (ii) results can be significant only in the long term.

Analyzing arthropods community structure emerged that some groups are worthy of attention in studying the effect of CCs on soil fauna. Indeed, leguminous-based cover crops were those with a community structure more similar to Control, while other CCs, such as R, induced changes in arthropods composition. Overall, Coleoptera was one of the most important taxa for discriminating between treatments, thus corroborating results by Vasconcellos *et al.* (2013) and Martins *et al.* (2018), which indicated that this order could be an efficient bio-indicator of soil quality. On the other hand, Hymenoptera, despite their lower abundance, was the group that more often influence treatments community dissimilarities, moreover their major abundance in VC, corresponding with the higher Pielou's evenness value, confirmed its role as indicator of other arthropod taxa changes (Menta and Remelli, 2020).

Nevertheless, C:N ratio and biomass input terms failed in building predictive models for arthropod-based variables. The reason could lie in the chosen variables on which we worked, since C:N ratio and biomass could affect arthropods depending on the trophic level to which they belong. An explanation that is grounded in Ebeling *et al.* (2014), where the abundance of decomposers was positively associated with increased plant biomass, whereas herbivore abundance increased with increasing C:N ratio. By altering parameters like root biomass and soil structure, CCs could affect soil biota food webs; for example van Eekeren *et al.* (2009) observed that with clover the availability of easily decomposable material in the rhizosphere and litter quality aspects, such as plant defensive compounds, may reduce bacterial and fungal biomass and the proportion of herbivorous nematodes, as well as increase the proportion of bacterivorous nematodes. Since those parameters directly affect some of the arthropod food habits, multiple mechanisms may combine to drive abundance and diversity patterns in mesofauna dynamics, suggesting that the introduction of grasses in CCs mixtures could be beneficial for promoting arthropods biodiversity.

CCs impact on soil fauna was more evident on earthworms, probably because organic materials are the main limiting factor for earthworm communities in cultivated sites (Pérès *et al.*, 2011). Earthworms higher abundance and biomass in RCC and VC further highlight the role of introducing leguminous species in a mixture for enhancing soil biology, as previously suggested by van Eekeren *et al.* (2009). For instance, these authors found that the introduction of clover in a grass sward often results into increased density and biomass of earthworm population and ascribed the reasons in the increased amount of above-ground dry matter production as residues. Moreover, as supported by the results obtained in this study, van Eekeren *et al.* (2009) also noted that the earthworm biomass had a negative relationship with the C:N ratio of residues, suggesting that the quality of residues – rather than the quantity – plays a key role in driving earthworm abundance. Finally, our results are also consistent with Shipitalo *et al.* (1988), who reported large weight gains in earthworms on diets of legumes, which had the lowest C:N ratio. So that, those results highlight the importance, especially in short-term studies, of integrate traditional biodiversity indexes with QBS-ar and community composition analysis, as well as different bioindicators, in order to have a broader view of the impact of agricultural management in soil dynamics.

5. Conclusions

Our 3-yr field study examined the effects of selected winter cover crops under NT on grain yield of maize and soybean, cumulative biomass, C, and N input to the soil, as well as soil C pools and biodiversity in a clay loam soil of a temperate region devoted to intensive crop production.

We observed that introducing cover crops for damping negative effects of transition (from conventional tillage to no-till) on crop yield and biomass is not always effective. Cover crop residues may indeed affect negatively plant biomass and grain yield in maize and soybean. Such an unfavorable effect could be avoided with cover crop residues with fast decomposition (as in our mixture hairy vetch plus crimson clover, which also allows to maximize N input) and erect posture after termination (as in our rye monoculture, in the case of early termination date).

In addition, we found that CCs need to be (also) targeted at producing residues with low C:N ratio (as that of our mixture phacelia plus white mustard), rather than only high rate of residues (i.e. high biomass and C input), in order to promote soil C cycling by enhancing total soil organic C and pools. For this reason, mixtures of selected cover crops species that allow the best compromise between a reasonable amount of residue and low residue C:N ratio should be preferred. Nevertheless, any change in soil C concentration and distribution seems to be limited the topmost 5 cm of soil.

The inclusion of extra-biomass amount into the soil with leguminous-based cover crops may also positively affect soil biodiversity. Our results suggested that properties of leguminous biomass could be considered efficient drivers to define the complexity of arthropod and earthworm communities. Nevertheless, most robust trend can be highlighted applying long-term studies.

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CHAPTER 3.

Long-term C and N sequestration under no-till is governed by biomass production of cover crops rather than differences in grass *vs.* legume biomass quality

This chapter is based on:

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Abstract

Agricultural activities through conventional and intensive practices contribute to climate change by increasing emission of reactive carbon (C) and nitrogen (N) from soils to the atmosphere. Minimum soil disturbance, appropriate residue management and cover cropping as conservation practices are perceived as key strategies to limit GHGs emissions by increasing soil C and N sequestration as soil organic matter (SOM), thus promoting soil aggregation and enhancing soil fertility. Yet, the actual contribution of conservation practices to C and N sequestration, as well as mechanisms behind chemical and biochemical stabilization of SOM in the long-term are still controversial.

In the present 9-year field study on a wheat-maize-soybean rotation we investigated the effect of no-till (NT) coupled with grass vs. legume cover crop (i.e., rye [*Secale cereale* L., NT-R] or hairy vetch [*Vicia villosa* Roth, NT-V]) on main crop yields, C and N input by cover and main crops, soil aggregation and C and N sequestration rates, in comparison with conventional tillage (CT). We hypothesized that NT-R may lead to higher biomass input, C sequestration and comparable yield to CT, while NT-V may increase N input, N sequestration and lead to comparable yield to CT.

We found that yield of winter wheat, maize, and soybean were never reduced under both NT treatments, neither during the transition phase, nor afterwards. Rye and hairy vetch provided the same amount of biomass and C input, although vetch doubled N input compared with rye. Moreover NT-V increased cumulative biomass and C input from main crop residues compared with NT-R. Both NT-R and NT-V promoted C (+0.4 Mg ha^{-1} y^{-1} and +0.6 Mg ha^{-1} y^{-1}, respectively) and N (+88 kg ha^{-1} y^{-1} and +145 kg ha^{-1} y^{-1}, respectively) soil sequestration, mainly due to the increase of macroaggregate-associated C and N, thus corroborating a major role of NT for macroaggregates formation and SOM stabilization within macroaggregates.

Since no difference was found between cover crops in terms of biomass input, and C and N sequestration potential, we concluded that cover crop biomass production (rather than biomass quality) and retention onto the soil as residue were the main drivers of soil C and N sequestration. Therefore, both rye and hairy vetch may be combined with NT and promise significant potential as effective C farming practices.

1. Introduction

Growing population, land degradation and climate change are significant threats to food security and sustainable human development in the near future (Godfray *et al.*, 2010; McNutt, 2013; Hossain *et al.*, 2020). Agricultural activities are among the main causes of climate change by contributing to 23% of the total anthropogenic greenhouse gas (GHG) emission (Shukla *et al.*, 2019). Nevertheless, increasing carbon (C) sequestration into agricultural soils have been identified as a significant tool to meet the ambitious goals of EU Green Deal for keeping under control the unfavourable effect of a changing climate (Dynarski *et al.*, 2020). Therefore, the so-called Carbon Farming should lead to a net CO_2 sequestration into the soil, while preserving soil health, playing a major role in the adaptation of agroecosystems to climate change, sustaining food availability and lowering fertilizer demand (Oliver and Gregory, 2015) to meet goals of the Farm-to-fork strategy by EU (European Commission, 2020) and the Sustainable Development Goals by FAO (Sachs, 2012) at the same time.

Soil organic carbon (SOC) and total nitrogen (STN) contents are often used as indicators to monitor soil health or quality (Cardoso *et al.*, 2013; Singh, 2018). Indeed, SOC regulates chemical, physical and biological processes in soil by affecting nutrient availability, water holding capacity, aggregation turnover and stability, and microbial activity (Herrick and Wander, 2018). Nitrogen (N) is instead the most important macronutrient for plant growth and metabolism (Mengel and Kirkby, 2001). Since the development of the Haber-Bosch process, N fertilizers are extensively used to address N deficiency in crops, leading to significant N losses especially when N inputs exceed plant needs or soil system capacity (Gruber and Galloway, 2008). Therefore, adopting innovative farming practices with the potential to concomitantly sequester C and N into the soil (as SOC and STN) is an effective way to increase soil fertility and lower the dependency of farmers on chemical fertilizers (Lal, 2004; Hansen *et al.*, 2017).

Regular return of fresh organic matter (from crop residues and/or manure) to soil has been indicated as a measure to enhance SOC and STN content, particularly when applied together with conservation tillage or no-till (NT), which minimize SOC and STN losses (Lal, 2015; Fiorini *et al.*, 2020a). The additional step is combining NT and cover crops (CCs) to further increase SOC and STN accumulation and conservation (Kong *et al.*, 2005; Ogle *et al.*, 2012; Boselli *et al.*, 2020). Nevertheless, different CCs may play different agro-ecological functions. Gramineous CCs are particularly recommended to enhance soil organic matter accumulation and C sequestration because of producing more biomass residue during and after termination for decomposers (Adetunji *et al.*, 2020), whereas legumes CCs maximize N input because of biological N-fixation, thus offering the opportunity to increase STN and reduce dependence on chemical N-fertilizers (Fiorini *et al.*, 2022).

No-till and CCs together may be helpful also for reducing aggregate turnover, thus increasing the residence time of C and N into the soil (Six *et al.*, 2002). It is well known that aggregates provide physical protection as well as chemical and biochemical stabilization to soil organic matter (SOM) by binding organic compounds to soil minerals and creating barrier between microorganisms and their substrate (Six *et al.*, 2000a, 2002). Tillage promotes physical disturbance to soil, thus increasing soil aggregates turnover and, as a consequence, C and N mineralization (Perego *et al.*, 2019). Yet, the real benefit of no-till for C sequestration, and therefore for climate change mitigation and soil fertility restoration, has been recently questioned (Powlson *et al.*, 2014; Du *et al.*, 2017). The main concern is that the increase of SOC stock in the most superficial layer is counteracted by the decrease of it in the deeper layers. In addition, the authors pointed out that the increase of C sequestration under NT may be short-term. To further complicate matters, contrasting information is available on selecting correct species of cover crops for SOC and STN sequestration: in fact, Poeplau and Don (2015) found that both legume and non-legume CCs have similar sequestration potential, whereas other studies suggest that legume CCs (Jian *et al.*, 2020) or grasses CCs (Fageria *et al.*, 2007) may sequester more C and N.

The main objective of the present study was to determine the effect of NT, combined with two different cover crops (rye [*Secale cereale* L.] and hairy vetch [*Vicia villosa* Roth]), on (i) crop yield during time; (ii) biomass, C and N input to the soil; (iii) C and N stabilization in soil aggregates along different soil layers; and (iv) C and N sequestration potential and efficiency, as compared with conventional tillage (CT) without CCs. We hypothesized that introducing conservation farming practices may enhance soil aggregation, thus providing stabilization of C and N. In particular, NT + rye (NT-R) may provide higher biomass input and therefore higher accumulation of SOC, while NT + vetch (NT-V) may increase STN and N stabilization into soil aggregates. Furthermore, we formulated the hypothesis that the effect on aggregation level and nutrient stabilization of NT is particularly pronounced in the topmost soil layer. Based on results by Boselli *et al.* (2020), an additional hypothesis was that no-till + CCs maintains main crop yield levels comparable to those of conventional tillage without CCs in the long-term (after a 5-yr transition period).

2. Materials and methods

2.1 Site description

We set up a nine-year field experiment at the CERZOO research farm in Piacenza (45°00′18.0″N, 9°42′12.7″E; 68 m above sea level), Po Valley, Northern Italy. The soil is a fine, mixed, mesic *Udertic Haplustalfs* (Soil Survey Staff, 2014), with a silty clay texture. The initial physico-chemical properties of soil in the top 0-30 cm layer were: organic matter 23 g kg^{-1}; pH in H_2O 6.8; bulk density 1.30 g cm^{-3}; sand 122 g kg^{-1}; silt 461 g kg^{-1}; clay 417 g kg^{-1}; STN (Kjeldahl) 1.2 g kg^{-1}; available P (Olsen) 32 mg kg^{-1}; exchangeable K (NH_4^+ Ac) 294 mg kg^{-1}; and cation exchange capacity 30 $cmol^+$ kg^{-1}. Meteorological data during the experiment were collected from an automatic station placed in the field. The site is characterized by a temperate climate (Cfa as Köppen classification), with an average annual temperature of 13.2 °C and annual rainfall of 839 mm, based on a 30-year average.

2.2 Experimental design

The experiment was established in autumn 2011 as a randomized complete block (RCB) with four replicates (blocks) and three treatments: (i) conventional tillage (CT; which included moldboard ploughing to 30-cm depth with crop residue incorporation and two rotary harrowing to 15-20 cm depth for seedbed preparation); (ii) no-till (NT; consisting of direct sowing on untilled soil with crop residue retained on the soil surface) plus rye (*Secale cereale* L.) as cover crop (NT-R); (iii) NT plus hairy vetch (*Vicia villosa* Roth) as cover crop (NT-V). Each plot was 22 m wide and 65 m long (1430 m^2). The seeding rate of CCs were 110 kg ha^{-1} for rye and 80 kg ha^{-1} for hairy vetch. Termination of CCs was conducted by spraying Glyphosate [N-(phosphonomethyl) glycine] at the rate of 3 L ha^{-1} about 14 days before seeding the following main crop (maize or soybean). During the nine-year trial, three courses have been iterated of the following rotation: winter wheat

(*Triticum aestivum* L. subsp. *aestivum*), maize (*Zea mays* L.) and soybean (*Glycine max* [L.] Merr.). Full details about soil-crop management are reported in Boselli *et al.* (2020).

2.3 Yield and biomass measurements

Aboveground biomass of CCs was assessed every year by manually harvesting three random areas of 3 m^2 in each plot right before termination. Yield components of main crops (total aboveground biomass, straw/stover and grain) were annually determined by randomly selecting and harvesting three areas of 6 m^2 in each plot. Thereafter, grain and biomass were manually separated, and dry matter yields were obtained by oven-drying sub-samples at 65 °C until constant weight. C and N concentrations were measured for all yield components by the Dumas combustion method with an elemental analyser (Vario Max CNS, Elementar, Germany).

Annual residue-derived C and N inputs to the soil (for both main and cover crops) were calculated by multiplying residue dry matter by C and N concentration. For 2013-2014, data on CCs are not available due to a severe slug attack during plant emergence, causing the failure of the cover crops. We calculated 9-yr cumulative biomass, C and N residue-derived input, and residue C:N ratio of main and cover crops as the sum of annual data. Average annual main crop yield was calculated, separately for the three crops (wheat, maize, and soybean), as the arithmetic mean of annual crop yield. In addition, we calculated average annual residue-derived C and N input from both main crops and CCs as the arithmetic mean of annual C and N input.

2.4 Soil sampling, analyses and calculations

Three soil samples were collected randomly from each plot at 30-cm depth in October 2020 after harvesting maize (9 years after no-till adoption). Each sample was divided into three layers: 0-5, 5-15, and 15-30 cm. The three samples of each depth section for each plot were combined and mixed together. As a result, four composite samples of each depth were collected for each treatment. Then, soil samples were air-dried and sieved at 8-mm diameter. Moreover, sub-samples were sieved at 2-mm diameter to determine SOC and N content using Dumas combustion method. Soil carbonates correction was not performed due to the absence of carbonates in the soil.

Soil aggregate size distribution analysis was performed on 8-mm diameter samples, according to Elliott (1986). In detail, 80 g of soil was submerged into deionized water for 5 minutes and wet sieved. Then, three sieves of 2000 µm, 250 µm, and 53 µm mesh were used to divide the four aggregate fractions: large macroaggregates (LM; >2000 µm), small macroaggregates (sM; 250-2000 µm), microaggregates (m; 53-250 µm) and silt-and-clay fraction (s+c; <53 µm). Each fraction was isolated by manually moving the sieve up and down 50 times during each phase (2 minutes). After each phase, soil aggregates remaining on the top of the sieve were transferred onto an aluminum pan, oven dried at 105 °C and weighed. Water and soil passing through the sieve were poured onto the smaller mesh sieve, thus starting the next phase.

We used the physical fractionation method developed by Six *et al.* (2000b) to isolate fractions within macroaggregates, namely coarse particulate organic matter (cPOM; >250 µm), microaggregates within macroaggregates (mM; 53-250 µm) and silt and clay (s+cM; <53 µm). Specifically, a composed subsample of LM and sM, in the same proportions obtained after wet sieving, was immersed in deionized water on top of a 250 µm sieve and sieved with 50 stainless steel beads (4 mm diameter) for 2 min. Once the macroaggregates had been cracked, organic fraction remaining on the top of the 250 µm was isolated and quantified as cPOM. Microaggregates and other released material passing through 250 µm ended up on 53 µm sieve and were sieved as in the wet sieving method. Soil aggregates retained by the sieve were isolated as mM, while those passed through the sieve as s+cM.

Correction for sand content was performed for all non-silt and clay fractions according to Elliott *et al.* (1991). C and N concentration of aggregate fractions was determined by using combustion method previously described.

The mean weight diameter (MWD) was calculated according to Van Bavel (1950) as follows:

$$MWD = \sum_{i=1}^{n} x_i \, w_i \quad (1)$$

where x_i is the mean diameter of each aggregate-size fraction separated by sieving, and w_i is the proportion of each sand-free aggregate-size fraction out of the entire sample weight.

Sampling for soil bulk density (BD) determination was performed along with soil sampling for aggregate stability assessment. BD was then calculated by dividing the dry weight of each soil layer (0-5; 5-15 cm; and 15-30 cm) by its volume.

Three undisturbed soil cubes of 8000 cm^3 (20 ×20 ×20 cm) were collected from each plot using a spade and immediately brought to the lab to assess earthworms abundance into the soil. Earthworms were manually separated from the soil and counted (Shepherd *et al.*, 2008). Thereafter, earthworms were weighted and oven-dried to determine dry biomass after voiding earthworm intestines according to Dalby *et al.* (1996). Then, earthworm density (number of earthworms per square meter) was calculated by multiplying the number of individuals and the dry biomass by 25.

2.5 Assessment of C and N sequestration rate in the soil

Soil organic C (SOC) and total N (STN) stocks (Mg ha^{-1} and kg ha^{-1}, respectively) were calculated for each soil layer by multiplying SOC or STN concentration, BD, and the depth of each soil layer. Then, SOC and STN stocks of the 0-30 cm soil layer were assessed as the weighted means of SOC and STN stocks of each soil layer. C and N sequestration (Mg ha^{-1} and kg ha^{-1}, respectively) in the soil was calculated as the difference between final (October 2020) and initial (October 2011) SOC and STN stocks of 0-30 cm soil layer. Average annual C and N sequestration rate (Mg ha^{-1} y^{-1} and kg ha^{-1} y^{-1}, respectively) was then calculated by dividing C and N sequestration by the duration of the experiment (9-yr). In addition, we calculated annual C sequestration efficiency (SE) as the ratio of average annual C sequestration and average annual C input. Average annual C input was considered as equivalent to the residue-derived C input from main crops and CCs.

2.6 Statistical analyses

We conducted a repeated measures analysis of variance (ANOVA) on main crop grain yield with soil management/CCs type, year, and block as fixed factors and replicate as random effect. Grain yield data were standardized using *Z-score* to perform repeated measures ANOVA because of crop diversity throughout the experiment. *Z-scores* were calculated as follows:

$$z = \frac{x - \mu}{\sigma} \quad (2)$$

where x is observed data point, μ is average grain yield for each year and σ is standard deviation.

A linear model was applied to study the effect of treatments on: (i) crop, CCs, and total biomass return (for each year and final); (ii) C and N input to the soil from both main crops and CCs (for each year and final); (iii) soil aggregate fractions, within-macroaggregates fractions and MWD; (iv) aggregate-associated C and N; and (v) C and N sequestration rates and C sequestration efficiency. Shapiro-Wilk and Levene's tests were performed to check normality and homogeneity of variances of measured variables. When the ANOVA assumptions were violated, data were log-transformed prior to analysis and back-transformed after the post-hoc test. Tukey's honestly significant difference (HSD) was used as *post-hoc* to test significant differences among treatments with a *p*-value of 0.05 as threshold.

Relationship between yields, residue-derived C/N inputs, soil aggregate stability parameters, soil biological activity (earthworms), and C/N sequestration parameters was assessed by performing two principal component analyses (PCA) with type II scaling separately for C and N parameters.

We used R 4.0.3. (R Core Team, 2020) with nlme (Pinheiro *et al.*, 2013), multcomp (Hothorn *et al.*, 2007), and FactoMineR (Lê *et al.*, 2008) packages for the linear model, mixed model, HSD test and PCA, respectively.

3. Results

3.1 Crop grain yield

Standardized grain yield of main crops was significantly affected by treatments in 2014, 2015, and 2016 (Figure 1). In detail, CT had higher *Z-score* than NT-R in 2014 with maize, while no differences were found between NT-V and other treatments. NT-R increased *Z-score* compared with CT and NT-V in 2015 with soybean (+14% and +17%, respectively). In 2016, winter wheat grain yield showed higher *Z-score* under CT and NT-V than under NT-R. Each treatment was affected by year (Figure 1): grain yield of CT was higher in 2014 than in 2012 and 2017; grain yield of NT-R was lower in 2016 than in 2015; and grain yield under NT-V was higher in 2018 than in 2020.

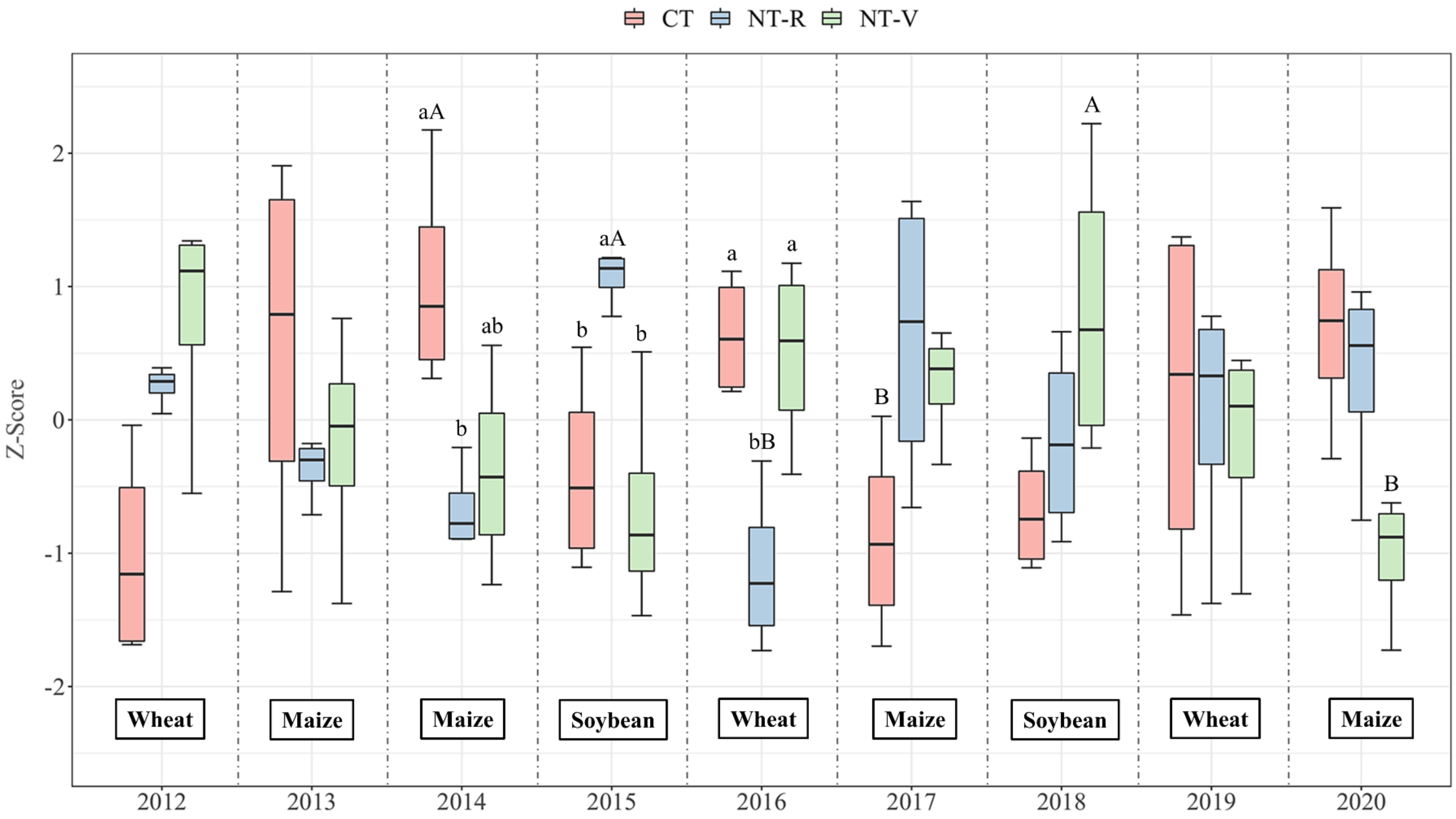

Figure 1. Box-plots of crop yield, reported as *Z-scores*, as affected by soil management (conventional tillage [CT], no-till + rye [NT-R], and no-till + hairy vetch [NT-V]) from 2012 to 2020. The bottom and top of each box represent the lower and upper quartiles respectively, the line inside each box shows the median and whiskers indicate minimal and maximum observations. Capital letters indicate differences among years within the same soil management; lowercase letters indicate differences among soil management within the same year (p-value < 0.05).

3.2 Biomass and residue-derived C/N input to the soil

Annual biomass input to the soil from main crop residues was never significantly affected by treatments (Table 1). However, 9-yr cumulative biomass input was higher under NT-V than under NT-R, whereas no difference was recorded between CT and NT treatments (Table 1). Similarly, only cumulative C input from main crop residues was affected by treatments, and NT-V increased cumulative C input compared with NT-R, while no differences were found between CT and NT treatments (Table 1). N input from main crops was higher under NT-V than under CT in 2016 (+42%) and with NT-R in 2018 (+27%) (Table 1). C:N ratio was affected by treatments in 2015, 2016, 2018, 2019, and as mean value (Table 1). In detail, NT-R increase C:N ratio compared with NT-V and CT in 2015 and in 2018, while both NT-R and NT-V reduced C:N ratio compared with CT in 2016, in 2019, and as mean value.

Biomass input of CCs was almost never affected by different CCs type except for 2020, when NT-V doubled biomass input compared with NT-R (Table 1). Same pattern was found for C input from CCs; indeed, NT-R reduced C input in 2020 (Table 1). N input from CCs was affected by treatments in 2013, 2018, 2020, and as cumulative rate (Table 1). NT-V increased N input from CCs by almost 50% in 2013, 63% in 2018, 365% in 2020, and 92% as 9-yr cumulative rate (Table 1). Finally, C:N ratio was consistently reduced by NT-V, ranging from 9 to 18, compared with NT-R (-46% as mean value) (Table 1).

Year	**Treatment**	**Main crop input**				**Cover crop input**			
		Biomass input	**C input**	**N input**	**C:N ratio**	**Biomass input**	**C input**	**N input**	**C:N ratio**
		Mg ha^{-1}	Mg ha^{-1}	kg ha^{-1}		Mg ha^{-1}	Mg ha^{-1}	kg ha^{-1}	
2012	**CT**	7.62	3.22	48.2	67.9	-	-	-	-
	NT-R	7.47	3.14	45.7	72.5	-	-	-	-
	NT-V	7.74	3.30	52.1	65.9	-	-	-	-
	***p*-value**	n.s.	n.s.	n.s.	n.s.	**-**	**-**	**-**	**-**
2013	**CT**	16.3	6.98	111	62.8	-	-	-	-
	NT-R	15.4	6.51	107	60.8	3.13	1.35	**55.9 b**	**24 a**
	NT-V	16.8	7.13	126	56.6	4.28	1.82	**103.4 a**	**18 b**
	***p*-value**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	**0.007**	**< 0.001**
2014	**CT**	11.5	4.95	68.7	72.1	-	-	-	-
	NT-R	11.0	4.65	75.2	62.4	-	-	-	-
	NT-V	11.5	4.88	70.6	69.9	-	-	-	-
	***p*-value**	n.s.	n.s.	n.s.	n.s.	**-**	**-**	**-**	**-**
2015	**CT**	3.85	1.58	38.2	**41.3 b**	-	-	-	-
	NT-R	3.46	1.46	29.3	**50.0 a**	3.12	1.34	47.7	**28 a**
	NT-V	3.31	1.39	31.7	**43.9 b**	2.09	0.89	67.7	**13 b**
	***p*-value**	n.s.	n.s.		**0.008**	n.s.	n.s.	n.s.	**< 0.001**
2016	**CT**	7.48	3.17	**36.1 b**	**87.8 a**	-	-	-	-
	NT-R	7.16	3.03	**44.3 ab**	**68.3 b**	-	-	-	-
	NT-V	7.88	3.36	**51.1 a**	**66.0 b**	-	-	-	-
	***p*-value**	n.s.	n.s.	**0.012**	**0.003**	**-**	**-**	**-**	**-**

2017	**CT**	9.56		4.10		82.9		51.8		-		-		-		-	
	NT-R	9.76		4.11		111.2		37.1		2.34		1.01		70.3		**14**	**a**
	NT-V	9.42		4.00		94.8		42.4		1.97		0.84		91.4		**9**	**b**
	***p*-value**	n.s.		n.s.		n.s.		n.s.		n.s.		n.s.				**< 0.001**	
2018	**CT**	3.86		1.58		**38.3**	**a**	**41.3**	**b**	-		-		-		-	
	NT-R	3.25		1.37		**27.5**	**b**	**50.0**	**a**	2.83		1.21		**52.5**	**b**	**23**	**a**
	NT-V	3.67		1.54		**34.8**	**a**	**44.1**	**b**	1.92		0.81		**85.4**	**a**	**10**	**b**
	***p*-value**	n.s.		n.s.		**0.003**		**0.006**		n.s.		n.s.		**0.046**		**< 0.001**	
2019	**CT**	9.66		4.09		46.9		**87.8**	**a**	-		-		-		-	
	NT-R	8.84		3.72		53.6		**69.5**	**b**	-		-		-		-	
	NT-V	10.28		4.39		66.3		**66.0**	**b**	-		-		-		-	
	***p*-value**	n.s.		n.s.		n.s.		**0.002**		**-**		**-**		**-**		**-**	
2020	**CT**	10.9		4.68		81.4		62.1		-		-		-		-	
	NT-R	11.6		4.88		102.8		50.9		**1.98**	**b**	**0.85**	**b**	**32.43**	**b**	**26**	**a**
	NT-V	12.0		5.10		103.7		53.1		**3.93**	**a**	**1.68**	**a**	**150.7**	**a**	**11**	**b**
	***p*-value**	n.s.		n.s.		n.s.		n.s.		**0.005**		**0.005**		**< 0.001**		**<0.001**	
Cumulative	**CT**	**80.8**	**ab**	**34.3**	**ab**	552		**62.4**	**a**	-		-		-		-	
	NT-R	**77.4**	**b**	**32.7**	**b**	592		**55.2**	**b**	13.4		5.76		**259**	**b**	**22***	**a**
	NT-V	**82.4**	**a**	**35.0**	**a**	630		**55.6**	**b**	14.2		6.04		**499**	**a**	**12***	**b**
	***p*-value**	**0.033**		**0.032**		n.s.		**0.034**		n.s.		n.s.		**< 0.001**		**< 0.001**	

* Mean values

Table 1. Biomass input (Mg ha-1), residue-derived carbon (C) input, residue-derived nitrogen (N) input, and C:N ratio of both main crops and cover crops as affected by soil management/CCs type. Conventional tillage (CT), no-till + rye (NT-R), and no-till + vetch (NT-V). Lowercase letters indicate differences among treatments within the same year.

3.3 Aggregate size distribution

Aggregate amounts were significantly affected by treatments in the 0-5 cm and in the 5-15 cm soil layers, whereas no difference was found in the 15-30 cm layer (Table 2). Indeed, LM were significantly lower under CT than under NT-R and NT-V in the topmost layer (-84% and -82%, respectively). Consequently, all other aggregates fractions in the 0-5 cm layer (sM, m and s+c) were reduced by NT treatments compared with CT. Conversely, CT increased LM by 18% compared with NT-R in the 5-15 cm soil layer, but reduced them by 17% compared with NT-V. Either sM, m and s+c were higher under NT-R than under NT-V in the 5-15 cm layer, whereas no difference occurred between CT and NT treatments.

Treatments significantly affected fractions within macroaggregates amount in the 0-5 cm and in the 5-15 cm soil layers. NT-R increased cPOM, mM, and s+cM compared with CT in the topmost layer, while no difference was found between NT-R and NT-V. Only mM was higher under NT-V than under CT in the 0-5 cm layer (+20%). Regarding the 5-15 cm soil layer, treatments affected only s+cM, which was increased under NT-V (289 g kg^{-1} soil) compared with NT-R and CT (234 g kg^{-1} soil both). NT-V had higher cPOM than NT-R and CT in the deepest soil layer, while NT-R even reduced cPOM compared with CT. MWD was higher under both NT treatments than under CT in the 0-5 cm soil layer, whereas NT-V increased MWD compared with NT-R and CT in the 5-15 cm layer.

Depth	**Treatment**	**Aggregate fractions** (g kg^{-1} soil)								**Aggregate fractions within macroaggregates** (g kg^{-1} soil)						**MWD** (mm)	
		LM[1]		**sM**[2]		**m**[3]		**s+c**[4]		**cPOM**[5]		**mM**[6]		**s+cM**[7]			
0-5 cm	**CT**	**51**	**b**	**620**	**a**	**226**	**a**	**103**	**a**	**37**	**b**	**406**	**b**	**229**	**b**	**0.88**	**b**
	NT-R	**314**	**a**	**492**	**b**	**120**	**b**	**73**	**b**	**50**	**a**	**486**	**a**	**271**	**a**	**2.33**	**a**
	NT-V	**291**	**a**	**505**	**b**	**130**	**b**	**73**	**b**	**47**	**ab**	**485**	**a**	**265**	**ab**	**2.21**	**a**
	***p*-value**	**< 0.001**		**0.022**		**0.004**		**0.004**		**0.015**		**0.008**		**0.035**		**< 0.001**	
5-15 cm	**CT**	**273**	**b**	**516**	**ab**	**140**	**ab**	**71**	**ab**	35		521		**234**	**b**	**2.10**	**b**
	NT-R	**231**	**c**	**535**	**a**	**153**	**a**	**81**	**a**	39		493		**234**	**b**	**1.87**	**b**
	NT-V	**331**	**a**	**490**	**b**	**118**	**b**	**61**	**b**	38		494		**289**	**a**	**2.43**	**a**
	***p*-value**	**< 0.001**		**0.048**		**0.039**		**0.032**		n.s.		n.s.		**0.025**		**< 0.001**	
15-30 cm	**CT**	326		477		123		74		**39**	**b**	524		240		2.39	
	NT-R	365		456		115		64		**26**	**c**	546		250		2.60	
	NT-V	352		462		128		58		**49**	**a**	507		257		2.53	
	***p*-value**	n.s.		n.s.		n.s.		n.s.		**< 0.001**		n.s.		n.s.		n.s.	

[1] LM: macroaggregates with a large size (>2 mm).

[2] sM: macroaggregates with a small size (2 mm - 250 µm).

[3] m: microaggregates (250 µm - 53 µm).

[4] s + c: silt and clay (< 53µm).

[5] cPOM: coarse particulate organic matter within macroaggregates (>250 µm).

[6] mM: microaggregates within macroaggregates (250 - 53 µm).

[7] s + cM: silt and clay within macroaggregates (< 53 µm).

Table 2. Aggregates soil distribution (g kg^{-1}; sand-free) acquired from wet sieving of whole soil and from macroaggregates in different soil layers (0-5 cm; 5-15 cm; 15-30 cm) and mean weight diameter (MWD; mm) as affected by soil management/CCs type after 9 years: conventional tillage (CT); no-till + rye (NT-R); no-till + vetch (NT-V). Lowercase letters indicate differences among treatments within the same soil layer. *P*-values are also reported for each soil layer and aggregate fraction.

3.4 SOC and aggregates-associated C

After 9 years of trial, soil organic C concentration was increased under NT-R and NT-V in both 0-5 and 5-15 cm soil layers, whereas no difference was found between treatments in the 15-30 cm soil layer (Table 3).

Similarly, C concentration in aggregates fractions was never affected by different treatments in the 15-30 cm soil layer (Table 3). CT reduced C content of LM and sM in the 0-5 cm soil layer compared with NT-R (-91% and -33%, respectively) and NT-V (-91% and -30%, respectively). In the 5-15 cm soil layer, C content associated with LM was higher under NT-V (4.87 g kg^{-1} soil) than under NT-R (3.08 g kg^{-1} soil) and CT (3.18 g kg^{-1} soil). On the other hand, NT-R increased C content of m and s+c compared with NT-V and CT in the 5-15 cm soil layer.

C associated with fractions within macroaggregates in the 0-5 cm soil layer was generally higher under NT-R and NT-V than under CT, although no difference was found between NT-V and CT for s+cM. In detail, NT-V and NT-R increased approximately fourfold C associated with cPOM and doubled C associated with mM. Conversely, in the 5-15 cm soil layer C concentration was affected by treatments exclusively for s+cM: NT-V increased C concentration compared with CT (+48%), while no difference occurred between NT-R and other treatments. NT-R had lower C associated with cPOM in the 15-30 cm soil layer compared with NT-V and CT, while the latter reduced C associated with s+cM compared with NT-V by 29%.

Depth	**Treatment**	**SOC**[1] (g C kg^{-1} soil)	**Carbon content in aggregates** (g C kg^{-1} soil)				**Redistribution of C within macroaggregates** (g C kg^{-1} soil)		
			LM[2]	**sM**[3]	**m**[4]	**s+c**[5]	**cPOM**[6]	**mM**[7]	**s+cM**[8]
0-5 cm	**CT**	**11.8 b**	**0.61 b**	**7.22 b**	2.44	1.54	**0.48 b**	**4.8 b**	**3.03 b**
	NT-R	**21.0 a**	**6.76 a**	**10.82 a**	2.00	1.43	**2.48 a**	**10.6 a**	**4.85 a**
	NT-V	**20.4 a**	**6.71 a**	**10.29 a**	2.17	1.19	**2.17 a**	**10.2 a**	**4.48 ab**
	p-value	**< 0.001**	**0.003**	**< 0.001**	n.s.	n.s.	**< 0.001**	**< 0.001**	**0.024**
5-15 cm	**CT**	**11.6 b**	**3.18 b**	6.12	**1.48 b**	**0.82 b**	0.43	6.2	**3.1 b**
	NT-R	**13.1 a**	**3.08 b**	7.17	**1.81 a**	**1.04 a**	0.66	6.7	**3.4 ab**
	NT-V	**14.3 a**	**4.87 a**	7.00	**1.57 b**	**0.88 b**	0.57	7.2	**4.6 a**
	p-value	**0.003**	**< 0.001**	n.s.	**0.009**	**0.010**	n.s.	n.s.	**0.041**
15-30 cm	**CT**	11.5	3.73	5.59	1.28	0.94	**0.50 a**	6.2	**2.9 b**
	NT-R	11.6	4.09	5.33	1.30	0.83	**0.26 b**	6.5	**3.1 ab**
	NT-V	11.7	4.18	5.39	1.43	0.74	**0.48 a**	5.9	**4.1 a**
	p-value	n.s.	n.s.	n.s.	n.s.	n.s.	**0.006**	n.s.	**0.038**

[1] SOC: soil organic carbon content.

[2] LM: macroaggregates with a large size (>2 mm).

[3] sM: macroaggregates with a small size (2 mm - 250 µm).

[4] m: microaggregates (250 µm - 53 µm).

[5] s + c: silt and clay (< 53µm).

[6] cPOM: coarse particulate organic matter within macroaggregates (>250 µm) .

[7] mM: microaggregates within macroaggregates (250 - 53 µm).

[8] s + cM: silt and clay within macroaggregates (< 53 µm).

Table 3. Soil organic C (SOC) content of the whole soil and associated with aggregate-size fractions in different soil layers (0-5 cm; 5-15 cm; 15-30 cm) as affected by soil management/CCs type after 9 years: conventional tillage (CT); no-till + rye (NT-R); no-till + vetch (NT-V). Lowercase letters indicate differences among treatments within the same soil layer. *P*-values are also reported for each soil layer and aggregate fraction.

3.5 STN and aggregates-associated N

Soil total N was increased by NT-R and NT-V compared with CT in both 0-5 and 5-15 cm soil layers, while treatments did not affect STN in the deepest layer (Table 4). Regarding the topmost soil layer (0-5 cm), N associated with aggregates was generally higher under NT-V and NT-R than under CT. In detail, both NT-R and NT-V enhanced N content in LM and in sM compared with CT, while m and s+c were not affected by treatments. NT-V enhanced N content of LM by 69% compared with both CT and NT-R in the 5-15 cm soil layer, while N associated with microaggregates was higher under NT-R (0.205 g kg^{-1} soil) than under CT (0.166 g kg^{-1} soil). Treatments did not affected N associated with aggregates in the 15-30 cm soil layer. N content of aggregates within macroaggregates was higher under NT-R and NT-V than under CT for all fractions (cPOM, mM and s+cM) in the 0-5 cm soil layer. In the 5-15 soil layer, cPOM and mM were not affected by treatments and NT-V enhanced N associated with s+cM compared with CT (+38%) and NT-R (+28%). In the deepest soil layer, NT-R reduced N content of cPOM compared with other treatments, while no differences were observed for mM and s+cM.

Depth	**Treatment**	**STN**[1] (g N kg^{-1} soil)		**Nitrogen content in aggregates** (g N kg^{-1} soil)							**Redistribution of N within macroaggregates** (g N kg^{-1} soil)					
				LM[2]		**sM**[3]		**m**[4]		**s+c**[5]	**cPOM**[6]		**mM**[7]		**s+cM**[8]	
0-5 cm	**CT**	**1.09**	**b**	**0.050**	**b**	**0.664**	**b**	0.260		0.121	**0.034**	**b**	**0.446**	**b**	**0.247**	**b**
	NT-R	**1.76**	**a**	**0.561**	**a**	**0.883**	**a**	0.209		0.109	**0.156**	**a**	**0.807**	**a**	**0.451**	**a**
	NT-V	**1.89**	**a**	**0.583**	**a**	**0.899**	**a**	0.250		0.159	**0.142**	**a**	**0.903**	**a**	**0.435**	**a**
	p-value	**< 0.001**		**0.002**		**0.002**		n.s.		n.s.	**< 0.001**		**< 0.001**		**0.002**	
5-15 cm	**CT**	**1.11**	**b**	**0.289**	**b**	0.542		**0.166**	**b**	0.111	0.028		0.621		**0.308**	**b**
	NT-R	**1.31**	**a**	**0.289**	**b**	0.674		**0.205**	**a**	0.142	0.040		0.633		**0.333**	**b**
	NT-V	**1.46**	**a**	**0.488**	**a**	0.677		**0.189**	**ab**	0.107	0.038		0.734		**0.425**	**a**
	p-value	**0.002**		**< 0.001**		n.s.		**0.021**		n.s.	n.s.		n.s.		**0.004**	
15-30 cm	**CT**	1.12		0.339		0.516		0.147		0.117	**0.038**	**a**	0.619		0.287	
	NT-R	1.16		0.397		0.490		0.159		0.112	**0.018**	**b**	0.718		0.356	
	NT-V	1.22		0.424		0.519		0.184		0.097	**0.037**	**a**	0.524		0.284	
	p-value	n.s.		n.s.		n.s.		n.s.		n.s.	**0.022**		n.s.		n.s.	

[1] STN: soil total nitrogen content.

[2] LM: macroaggregates with a large size (>2 mm).

[3] sM: macroaggregates with a small size (2 mm - 250 µm).

[4] m: microaggregates (250 µm - 53 µm).

[5] s + c: silt and clay (< 53µm).

[6] cPOM: coarse particulate organic matter within macroaggregates (>250 µm) .

[7] mM: microaggregates within macroaggregates (250 - 53 µm).

[8] s + cM: silt and clay within macroaggregates (< 53 µm).

Table 4. Soil total N (STN) content of the whole soil and associated with aggregate-size fractions in different soil layers (0-5 cm; 5-15 cm; 15-30 cm) as affected by soil management/CCs type after 9 years: conventional tillage (CT); no-till + rye (NT-R); no-till + vetch (NT-V). Lowercase letters indicate differences among treatments within the same soil layer. *P*-values are also reported for each soil layer and aggregate fraction.

3.6 C and N sequestration parameters

No-till treatments increased C sequestration rate and efficiency compared with CT, with no significant difference between them (Figure 2).

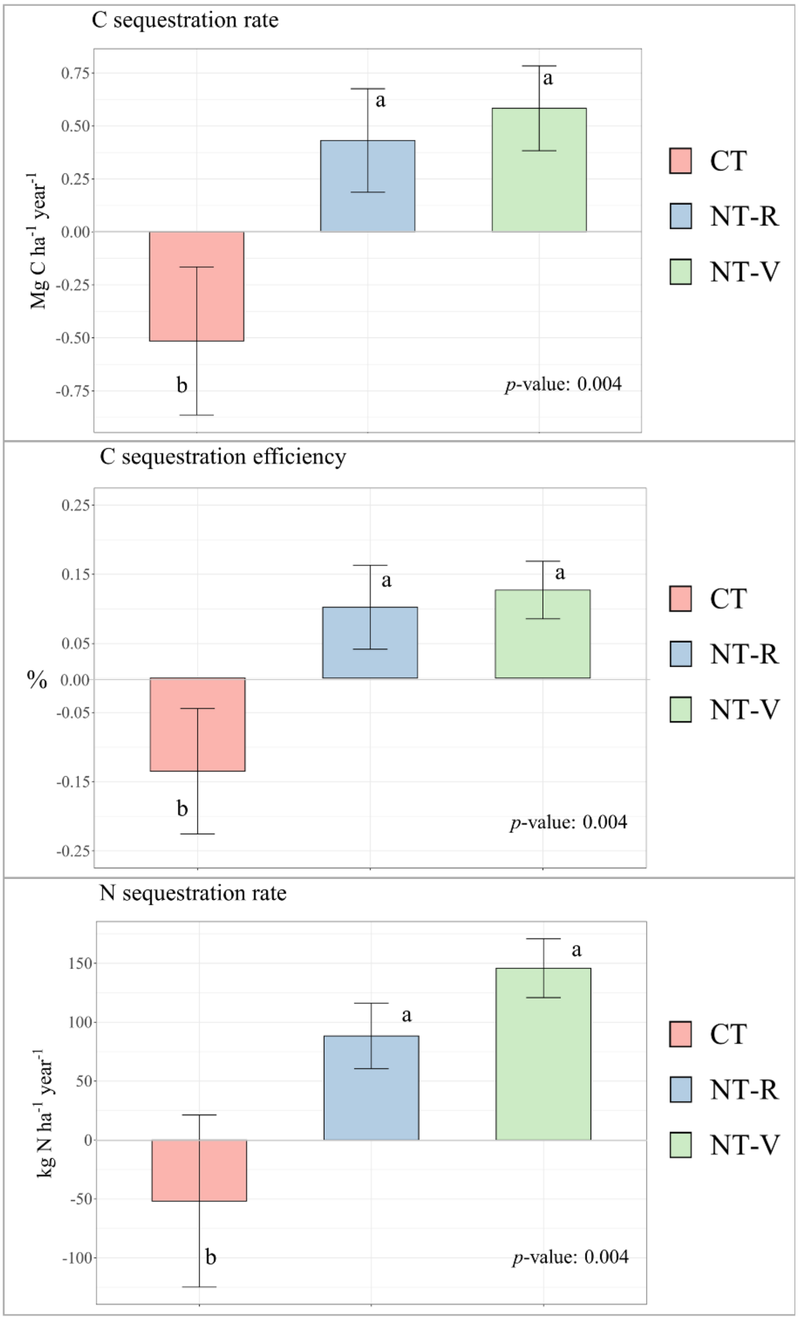

Figure 2. C and N sequestration rates and C sequestration efficiency as affected by soil management (conventional tillage [CT], no-till + rye [NT-R], and no-till + hairy vetch [NT-V]) after 9 years of experiment. Mean values ± standard deviation. Letters indicate differences among soil management. *p*-values are reported.

In detail, almost 0.6 Mg ha^{-1} of C were sequestered each year in the 0-30 cm soil layer of NT-V and slightly more than 0.4 Mg ha^{-1} for NT-R. Conversely, CT reduced C stock by about 0.5 Mg ha^{-1} y^{-1}. NT-V and NT-R stored the 13% and the 10%, respectively, of annual C input provided as residues, both significantly higher than CT (-0.13%).

N sequestration rate follows the same pattern as for C (Figure 2). Indeed, we did not find significant differences between the two NT treatments; but, NT-R and NT-V both increased N sequestration rate of 0-30 cm soil layer compared with CT. Specifically, N sequestration rate was 88 kg ha^{-1} y^{-1} for NT-R and 146 kg ha^{-1} y^{-1} for NT-V, while CT lost more than 50 kg ha^{-1} each year.

3.7 Relationships between variables

The first two principal components of PCA (Dim1 and Dim2) related to C parameters accounted for the 64.6% of the total variance (Figure 3a). C input from main crops, crop yields and C associated with s+c fraction were the variables which contributed the least to the total variation. Conversely all other variables were found to greatly contribute to total variability (more than 6%). C sequestration parameters were positively related with C input from CCs and with C associated with large aggregate fractions (LM and sM) as well as with fractions within macroaggregates (cPOM, mM, and s+cM). In addition, C sequestration rate and efficiency were negatively related with C:N ratio and – partially – positively related to earthworms abundance and MWD.

The first two principal components of PCA (Dim1 and Dim2) related to N parameters explained the 69.3% of the total variation (Figure 3b). Similarly to C, crop yields were the worst variables in terms of contribution to total variation (less than 4%). N sequestration rate was positively correlated with N associated with cPOM and LM, and partially with MWD and N input from CCs. In addition, a weak positive correlation was found between N sequestration rate and N associated with mM and with sM, as well as earthworms. Similarly to previous PCA, C:N appears to be negatively correlated with sequestration parameters.

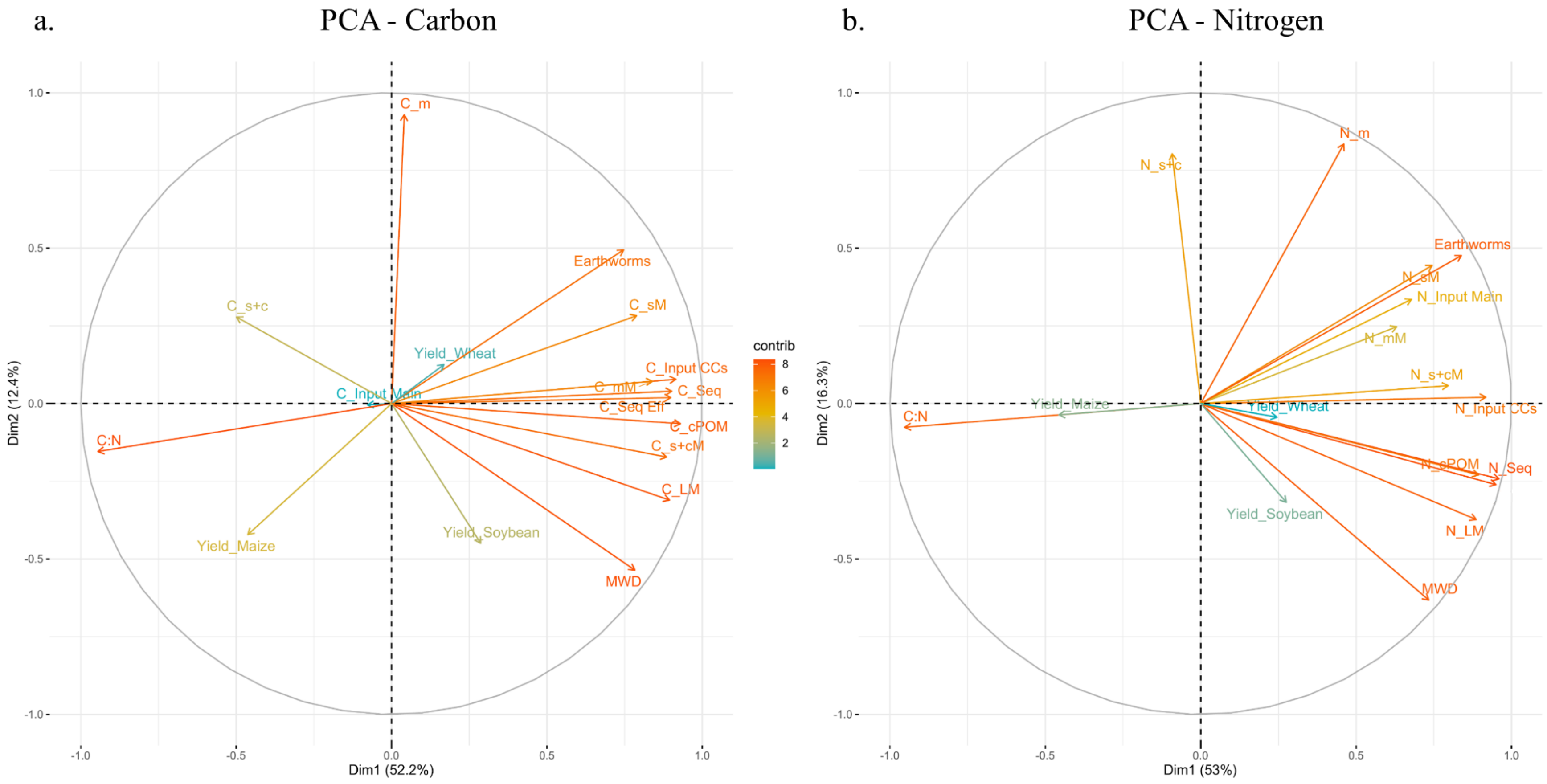

Figure 3. Principal Component Analyses of C- (a) and N-related parameters (b). The contributions of variables in accounting for the variability in a given principal component are expressed in percentage.

4. Discussion

4.1 Crop yield, biomass return, C and N inputs to the soil as affected by soil management

Grain yield of main crops was sometimes moderately affected by NT and cover crops, but without a specific pattern among treatments during the 9-years experiment. As reported previously by Boselli *et al.* (2020), conversion to NT plus CCs did not drastically reduce the yield of wheat, maize, and soybean compared to CT during the transition period (first 5 years of experiment). In detail, yield was higher under CT than under NT-R in 2014 with maize, but not than under NT-V. A similar pattern was observed in 2016 with winter wheat, while NT-R outyielded both NT-V and CT in 2015 with soybean. The reduced grain yield of maize in 2014 and of wheat in 2016 under NT-R was likely due to the high C:N ratio of rye residues maintained on the soil surface and to the higher soil bulk density compared with CT. In fact, retaining cover crop residues with high C:N ratio on the soil surface are well known to increase soil N-immobilization (Malhi *et al.*, 2001; Burgess *et al.*, 2002; Jin *et al.*, 2008), thus limiting N availability for the following – high N-demanding – crop. In addition, higher bulk density of NT soil compared with CT was previously reported by Fiorini *et al.* (2018) in the same field. Consequently, the combination of these two factors may have limited maize and wheat performances. Results on crop yield in 2014 and 2016 are in agreement with those reported in the global meta-analysis conducted by Pittelkow *et al.* (2015), which found an overall 5.1% reduction in crop yield due to NT across almost 700 studies.

Conversely, the higher grain yield of soybean under NT-R than under CT and NT-V in 2015 was probably because of two main factors: (i) the longer persistence of rye residues on the soil surface due to high C:N ratio, which may have helped to preserve soil moisture during early stages of plant growth; and (ii) the reduced weed pressure and competition thanks to both physical effect of the rye mulch and release of allelopathic compounds. These explanations are further supported by previous studies, which reported allelopathic weed control of gramineous cover crops (Schulz *et al.*, 2013; Tabaglio *et al.*, 2013) and increased soybean grain yield after NT + gramineous CCs adoption because of reduced weed pressure and competition (Williams *et al.*, 2000). In addition, since legumes main crops – such as soybean – can meet a large part of their N demand through biological

N-fixation (Liu *et al.*, 2011), the negative effects of N-immobilization into the soil on plant growth and performances were minimised.

Grain yield was then more homogeneous between treatments in the last four years of our trial. This suggests that building fertility and restoring soil functions over time through NT and CCs synergic effect may be considered pivotal for maintaining crop yield in the long-term and offset potential negative effects of tillage ceasing.

Although annual biomass and residue-derived C inputs of main crops were never affected by different soil management, cumulative rates were higher under NT-V compared with NT-R, while CT was in the between. As previously described for grain yield, relatively high C:N ratio of rye residues may have caused a slightly decrease in the annual biomass production by the N-demanding crops (Ruffo *et al.*, 2004). This is supported by our findings on the higher C:N ratio of rye residue (22, on average) compared with that of vetch (12, on average), which may have promoted N immobilization into the soil leading to a lower biomass production. Moreover, residues with high C:N ratio are known to be more persistent on the soil surface because of N-immobilization (Bengtsson *et al.*, 2003; Fiorini *et al.*, 2020), which limits mineralization rate of residues (Frankenberger and Abdelmagid, 1985), thus lowering soil temperature (Teasdale and Mohler, 1993) and possibly delaying plant emergence as well as slowing early growth (Wang *et al.*, 2012). On the contrary, vetch residue neither reduced nor increased biomass and C input from main crop compared with CT, despite having a low C:N ratio and providing almost 500 kg N ha^{-1} in nine years. This was because of the relatively high N fertilization rate to maize and winter wheat in our agro-ecosystem (> 200 kg N ha^{-1} yr^{-1}). Indeed previous studies reported increased main crop performances after hairy vetch termination mainly with reduced (or zero) application of N-fertilizer rates (Miguez and Bollero, 2005; Marcillo and Miguez, 2017; Pott *et al.*, 2021), which was not the case in our study.

Regarding biomass input from CCs, vetch provided 14.2 Mg ha^{-1} of biomass input as cumulative rate, while NT-R 13.4 Mg ha^{-1}. These results are in contrast with our initial hypothesis and with a recent review performed by Ruis *et al.* (2019) reporting generally higher biomass production of rye compared with hairy vetch in temperate ecoregions. However, our findings could be justified by an early seeding timing and a late termination of the cover crops. In fact, Liebman *et al.* (2018) found higher biomass production (+3

Mg ha^{-1}) of hairy vetch when terminated in mid-May rather than in late April. This is because hairy vetch growth is particularly enhanced in spring when air temperature reaches 20 °C (Zachariassen and Power, 1991), while rye is well known to be adaptable to harsh temperatures (Stoskopf, 1985). Nonetheless, residue-derived N input was found affected by treatments: in fact, vetch provided about twice nitrogen compared with NT-R (499 kg N ha^{-1} vs 259 kg N ha^{-1}, respectively). Higher N concentration in vetch residue is related to the biological N fixation associated with N-fixing root symbioses of legumes (Larue and Patterson, 1981).

4.2 Soil aggregates, aggregate-associated C and N, and responses of SOC and STN

Reducing soil disturbance and enhancing biomass input to the soil with adoption of NT plus CCs may facilitate the formation and the preservation of water-stable macroaggregates (Bhattacharyya *et al.*, 2012). Consistently with our hypothesis, NT treatments enhanced aggregate formation and stabilization in the 0-5 cm soil layer, as demonstrated by our results on MWD. In this case, the major contribution to the increased MWD under NT-V and NT-R was given by the increase in large macroaggregates, which were almost fivefold higher than under CT. Conversely, small macroaggregates, free microaggregates, and silt+clay fractions were found higher under CT than under NT treatments, indicating that intensive tillage operations promote large macroaggregate breakdown into smaller fractions. Moreover, hairy vetch and rye residues under NT treatments provided additional fresh organic material to the soil compared with CT. The decomposition of such material by soil micro- and macro-fauna is related to the release of polysaccharides and organic acids, which are the binding agents involved in aggregate formation and stabilization (Tisdall and Oades, 1982; Decaëns, 2000). Avoiding soil disturbance and returning fresh organic material to the soil slowed the aggregation turnover rate, promoting the formation of stable large macroaggregates (Six *et al.*, 2000a; Álvaro-Fuentes *et al.*, 2009). This findings are in agreement with previous results obtained by Sheehy *et al.* (2015) in *Vertic Cambisol* and in *Eutric Regosol*, reporting higher LM under NT than under CT and increased sM amount under CT in 3 out of 4 study sites. Our results corroborated also previous outcomes by Pareja-Sánchez *et al.* (2017) in a semiarid Mediterranean climate and *Typic Xerofluvent*, which observed higher amount of water-stable sM under CT than under NT in 2 sampling dates out of 4, whereas no differences were recorded in the remaining dates. In addition, increased proportion of m and s+c due to macroaggregate breakdown after tillage operations have been also widely reported in literature (Six *et al.*, 1999; Six *et al.*, 2000a; Denef *et al.*, 2001; Bossuyt *et al.*, 2002; Grunwald *et al.*, 2016; Gao *et al.*, 2019).

Here for the first time, we show that combining hairy vetch as CC with NT increases soil aggregate stability down to 5-15 cm soil layer, even compared with rye plus NT. In fact, MWD and LM were higher under NT-V than under CT and NT-R. These results may be ascribed to the higher main crop residue (and C input from such residue as well) provided to the soil under NT-V and to the combination between no-till and different root architecture of hairy vetch and rye. Under NT, CCs residues are retained on the soil surface in addition to residues from main crops and, therefore, downward movement of organic material to deeper soil layers is mainly limited to macrofauna activity (e.g. earthworms). This means that SOM is increased especially in the topmost soil layer (up to 5-10 cm depth) under NT as observed in several studies (Kern and Johnson, 1993; Koch and Stockfisch, 2006; He *et al.*, 2011). However, despite grasses generally having greater fibrous root mass and volume than legumes (Haynes, 1980), most of the latter have prominent taproots allowing them to explore deeper soil layers (Sheaffer, 1989). Therefore, the increased macroaggregate proportion in the shallow soil layer of both NT treatments is probably promoted by the aboveground residues of CCs left on the soil surface, whereas root residues of hairy vetch left into the soil after termination may have provided fresh organic material for LM formation and stabilization in the 5-15 cm soil layer. No-till + CCs benefits on aggregate stabilization were not observed in the deepest soil layer (15-30 cm). This is in agreement with previous findings reported by a long-term study conducted by Sithole *et al.* (2019), in which the authors did not find differences in LM amount between CT and NT at 20-30 cm depths. Fractions within macroaggregates were found to differ especially in the 0-5 cm soil layer. Coarse particulate organic matter was increased by NT-R and microaggregates within macroaggregates were higher under both NT treatments than under CT. This confirm that soil disturbance increase macroaggregate turnover rate, inhibiting the formation of microaggregates within macroaggregates, which are known to be key elements to long-term C sequestration (Six *et al.*, 1998; Denef *et al.*, 2004).

Continuous no-till and cover cropping enhanced SOC and STN in both 0-5 and 5-15 cm soil layers, while not in the 15-30 cm one. The effect of NT and CCs was particularly pronounced in the 0-5 cm layer, in which SOC was increased by 78% under

NT-R and by 73% under NT-V compared with CT. Our findings are widely supported by previous research reporting higher SOM content in untilled soils than in conventional tilled soils (Arshad *et al.*, 1990; Six *et al.*, 2000a; Plaza-Bonilla *et al.*, 2010; Sapkota *et al.*, 2012). In our study, the greatest accumulation of SOC and STN in the 0-5 cm layer under NT treatments is explained firstly by the larger input of fresh organic residues (because of cover cropping), and secondly by the higher protection from mineralization of SOM within soil aggregates. SOM is protected within macroaggregates because of two main mechanisms: (1) labile SOM (e.g. cPOM) is short-term physically protected (until macroaggregate disruption) by creating physical barriers between microorganisms and enzymes and their substrates (Beare *et al.*, 1994; Six *et al.*, 2002); (2) stable SOM is long-term physically and chemically protected in microaggregates within macroaggregates of which formation is promoted by macroaggregate stability and organic substances availability (Six *et al.*, 2000a; Bandyopadhyay *et al.*, 2010). This is supported by our results on redistribution of C and N within macroaggregates in the 0-5 cm soil layer: both cPOM and mM-associated C and N were higher under NT treatments than under CT, thus corroborating higher protection for labile (cPOM-associated) and more stable (mM-associated) SOM.

In the 5-15 cm soil layer, despite NT treatments having higher SOC and STN than CT, results on aggregate-associated C and N were less affected by treatments. In contrast to our hypothesis, no difference in terms of SOC and STN was found between the two NT+CCs treatments in all soil layers, but NT-V increased LM-associated C and N whereas NT-R had more C stored in microaggregates and in silt+clay fraction. This was probably again due to different root architecture of hairy vetch and rye (as previously discussed for aggregate fractions) and to the higher main crop residue provided to the soil under NT-V, which favoured the cementation of organic substances and soil particles to form macroaggregates (Decaëns, 2000). Last but not least, legume cover crops were reported to increase the amount and lability of residue inputs, thus favouring organomineral associations into the soil and further enhancing C protection within aggregates (Veloso *et al.*, 2019).

4.3 C and N sequestration potential of no-till plus cover crops

Consistently with our initial hypothesis, NT plus CCs increased C and N sequestration rates and C sequestration efficiency. On average NT treatments sequestered around 0.5

Mg C ha^{-1} y^{-1} and 115 kg N ha^{-1} y^{-1}, emphasising the importance of conservation practices for climate change mitigation and soil fertility restoration. Indeed, CT lost around 0.5 Mg C ha^{-1} y^{-1} and 50 kg N ha^{-1} y^{-1}. The negative performance of CT in terms of C sequestration potential is emphasized by the results on C sequestration efficiency: CT, in fact, lost around 14% of the total annual C input. On the contrary, NT treatments sequestered on average 11% of the total annual C input. Such highly positive results on C and N sequestration potential and C sequestration efficiency of NT may be explained by the larger return of crop residues and by the enhanced aggregate formation and stabilization, thus limiting losses through mineralization (Six *et al.*, 2000a). This is supported by our results showing strong correlation between C and N within macroaggregates, and associated with cPOM and mM as well, and sequestration parameters. Our results significantly argue outcomes from a recent meta-analysis performed by Powlson *et al.* (2014), in which the authors reported limited benefit of no-till for climate change mitigation mainly due to specific issues: (i) the increase of SOC in the topmost soil layer under NT would be counteracted by a decrease in the deeper layer (20-40 cm), meaning no net increase in SOC stock; (ii) the most of studies reporting positive effect under NT have focused on SOC concentration rather than on SOC stock, thus not considering the changes in soil bulk density. In addition, the authors reported a value of 0.3 Mg C ha^{-1} y^{-1} as a standard accumulation rate for NT in their study; yet they considered it very optimistic and overestimated. In our study, soil accumulated almost 0.6 Mg C ha^{-1} y^{-1} under NT-V and around 0.4 Mg C ha^{-1} y^{-1} under NT-R in the 0-30 cm soil layer, thus confirming the potential of conservation practices for C sequestration in fine-textured soils and temperate climates. However, we agree that more long-term studies are needed to fully assess the prolonged effect of NT and cover crops on climate change mitigation and, possibly, assessing the effect of periodic tillage events (e.g., subsoiling), which are occasionally used as practice to reduce possible NT unfavourable effect (Conant *et al.*, 2007; Powlson *et al.*, 2012). Also, in contrast to our hypothesis, no differences were found between rye and hairy vetch in terms of C sequestration rate. This may be explained by the same amount of biomass and C input provided by both cover crops (Poeplau and Don, 2015), which is supported by the positive correlation between C sequestration parameters and C input from CCs. Thus, cover crop biomass production rate (rather than biomass quality) and retention onto the soil as residue was the main driver of soil C and N sequestration in our study.

4.4 Implications for efficient C farming via no-till plus cover crops adoption

Based on findings reported here, conservation practices – such as no-till + CCs – may be adopted to sustain yield performances (even during the transition period), while providing more C and N inputs to the soil (and C and N sequestration) compared with conventional tillage practices in fine-textured soils of temperate areas. However, particular attention should be paid with high N-demanding main crops (e.g., maize) due to N-immobilization into the soil, leading to N-deficiency during the initial stages of crop growth and yield losses. This could be particularly the case when adopting a grass (as rye, in our experiment) as previous CC may promote N immobilization further than what expected. On the other hand, legume CCs have the potential to provide notable N input to the soil. However, the effect on main crop yield may be significant only in N-limited environments. When N-fertilization rate is relatively high (>200 kg N ha^{-1} y^{-1}, as for intensive agro-ecosystems with high-yielding crops), additional N inputs from legume CCs mismatching plant absorption capacity may results in significantly higher N losses as nitrous oxide (N_2O) emissions (Fiorini *et al.*, 2020b). Indeed, C sequestration is not the only driver for assessing the contribution to climate change mitigation, and N_2O is recognized as a potent greenhouse gas with a global warming potential 273 times greater than that of CO_2 on a 100-year time horizon (de Haas and Andrews, 2022). Thus, further studies focusing on different levels of N-fertilization are needed to assess optimal balance between CC-derived N inputs and N-fertilizer rates, thus aiming to avoid surplus and losses.

Fiorini *et al.* (2020) already reported with data from the same field that NT *vs.* CT may concretely reduce N_2O emissions from intensive agro-ecosystems, while maintaining yield. Nevertheless, CC type was considered as a main driver of N_2O emissions in this study, since rye reduced N_2O compared with hairy vetch by 20-36%, because of the lower availability of soil mineral N (which is likely to have reduced also losses via leaching). This may partially explain also the lack of differences between rye and vetch in terms of N sequestration rate and efficiency in the present study. Indeed, a part of the N input provided by hairy vetch residues (which was higher under NT-V than under NT-R) may have been lost via NO_3^- leaching and/or N_2O emissions. Therefore, exceeding N inputs results neither into increased yield performance, nor into increased N storage into the soil.

Overall, both rye and hairy vetch combined with NT showed promising results for climate change mitigation and soil fertility restoration, with the potential of maintain crop yield even in intensively managed agro-ecosystems. Yet, we recommend that N fertilizers should be applied considering the expected rate and timing of available N from mineralization of different types of CC residues.

In conclusion, the adoption of no-till plus selected cover crops may be a viable C farming strategy to meet the ambitious goals of EU Green Deal and of Sustainable Development by FAO, promoting CO_2 sequestration into agricultural soils, sustaining food production while lowering fertilizer demand.

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CHAPTER 4.

Matching crop row and dripline distance in subsurface drip irrigation increases yield and mitigates N_2O emissions

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Abstract

Intensive irrigation and nitrogen (N) fertilization are often linked to low N-fertilizer efficiency, and to high emissions of the greenhouse gas nitrous oxide (N_2O). Efficient irrigation systems (e.g. subsurface drip irrigation [SDI]) combined with N-fertigation in a no-till agroecosystem can promote N-use efficiency, thereby curbing N_2O emissions without depressing crop yield. Yet, crop type and SDI plant settings (and management) such as dripline spacing may determine the agronomic and environmental performance of SDI. In this two-year field study on maize (*Zea mays* L.) - soybean (*Glycine max* [L.] Merr.) rotation with conservation agriculture management (no-till and cover crops), we investigated the effects of three different irrigation systems (SDI with a narrow dripline spacing (70 cm), SDI with a large dripline spacing (140 cm), and sprinkler irrigation [SPR]) on yield, N-fertilizer efficiency, and N_2O emissions in a fine-textured soil. We hypothesized that SDI systems (especially with narrow dripline distance) would increase yield and mitigate N_2O compared with SPR, and particularly for maize due to its higher water and nutrient demand. We found that SDI may increase maize yield (+31%) and N-fertilizer efficiency (+43-71%). These positive results were only observed during the drier year in which irrigation supplied ca. 80% of maize water requirements. The narrower dripline spacing mitigated N_2O emissions compared with sprinkler irrigation (by 44%) and with the wider spacing (by 36%), due to a more homogeneous distribution of N in soil, and to a lower soil moisture content. Soybean yield and N-use efficiency were not affected by the irrigation systems. Overall, our study provides important insights on key management decisions that define the sustainability of novel irrigation systems, and in particular SDI with a 70 cm dripline distance should be promoted for maize to increase productivity and decrease N_2O emissions in fine-textured soils.

1. Introduction

Nitrous oxide (N_2O) is a potent greenhouse gas with a global warming potential 273 times greater than that of CO_2 on a 100-year time horizon (Allan *et al.*, 2021), and is the most dominant ozone-depleting substance of the 21st century (Ravishankara *et al.*, 2009). Agriculture is the largest source of anthropogenic N_2O emissions (Montzka *et al.*, 2011; Syakila and Kroeze, 2011). Although N_2O production in soils may occur through several biotic and abiotic processes such as nitrifier denitrification (Wrage-Mönnig *et al.*, 2018), co-denitrification (Spott and Florian Stange, 2011), chemodenitrification (Van Cleemput, 1998), and dissimilatory nitrate reduction to ammonium (Rütting *et al.*, 2011), two processes are considered the main sources: nitrification (Skiba and Smith, 1993) and denitrification (Firestone *et al.*, 1980). All these processes may be considerably affected by agroecosystem management practices, including application of N-fertilizers, irrigation system, as well as crop type and residue management (Snyder *et al.*, 2007; Perego *et al.*, 2016; Wagner-Riddle *et al.*, 2017; Lin and Hernandez-Ramirez, 2020).

Conventional irrigation techniques (e.g. furrow and sprinkler [SPR]) combined with a single application of N-fertilizer at a high rate are known to boost N_2O emission (Mehmood *et al.*, 2019). This is mainly due to the simultaneous high moisture and mineral N availability in the soil, promoting N_2O emissions from nitrification and denitrification (Tian *et al.*, 2017; Millar *et al.*, 2018). This combination of practices is widespread in conventional agricultural systems due to easy implementation (Bierman *et al.*, 2012; Ayyub *et al.*, 2019). Yet, it can also lead to high N losses through leaching because of the mismatch between soil N availability and plant uptake in some pedoclimatic conditions and crop stages (Black *et al.*, 1985; Grant *et al.*, 2012; Xia *et al.*, 2017), thereby reducing N-use efficiency.

Micro-irrigation systems (i.e. surface and subsurface drip irrigation [SDI]) combined with split N-fertilization through fertigation have been suggested as a measure to reduce N_2O emissions from soils and increase N-use efficiency (Li *et al.*, 2018; Sandhu *et al.*, 2019; Kuang *et al.*, 2021). This is mainly a consequence of partial soil wetting and enhanced plant N-uptake throughout the growing season (Kallenbach *et al.*, 2010; Mehmood *et al.*, 2019). Particularly, SDI can further reduce N losses compared with surface drip irrigation by optimizing spatial N-fertilizer application (as released near the rhizosphere), and decreasing surface soil wetting (Kallenbach *et al.*, 2010; Maris *et al.*, 2015; Wei *et al.*, 2018). However, recent studies reported higher N_2O emissions under micro-irrigation systems compared with conventional methods because of more frequent soil drying-wetting cycles, which increases soil N mineralization rates (Kuang *et al.*, 2018). These inconsistencies emphasize the need for further investigations.

Crop type/sequence is recognized as a major driver of agro-environmental performance. For instance, the response of yield, N-use efficiency (NUE) and N_2O emissions to different irrigation and fertilization systems vary strongly depending on the crop physiological groups (e.g. Gramineae vs. leguminous plants). Indeed, since leguminous crops such as soybean can meet a large part of their N demand through biological N fixation, they often do not require N fertilization (Liu *et al.*, 2011), leading to lower N_2O emissions than non-leguminous crops (Schmeer *et al.*, 2014). Moreover, Gramineae species with C4 photosynthetic pathway (e.g. maize) have higher water and N-use efficiency compared with C3 species such as soybean (Ghannoum *et al.*, 2010). Accordingly, the potential benefits of micro-irrigation systems for improving yield and N-use efficiency may be more pronounced for maize than for soybean.

In SDI systems, dripline spacing affecting dynamics of irrigation water distribution is one of the key management decisions with potential consequences for N losses, crop yields, and N-use efficiency. Dripline distance is usually set as an integer multiple of the crop row spacing, which may vary depending on crop-soil variables, and ranges between 70 and 300 cm (Lamm *et al.*, 1997; Lamm, 2016; Lee *et al.*, 2018). By lowering the amount of water and N per unit of soil volume delivered to the rooting zone at each irrigation, an appropriate dripline spacing to match the crop row spacing (e.g. 70 cm in maize) could further improve water and N spatial distribution compared to wider dripline spacing. On an area basis, it could be argued that a wider spacing could create larger dry areas, thus reducing area-scaled N_2O emissions. However, considering the non-linear relationship between N_2O emissions and water and N availability (Davidson, 1991; Kim *et al.*, 2013; Shcherbak *et al.*, 2014), the N_2O hotspots in the wet areas with wide dripline spacing (with much higher water and N application per unit of soil volume) could be much greater than the low fluxes from the dry areas generated with this system. Therefore, reducing dripline distance may increase yields and NUE as well as reduce N losses, although this also implies higher system costs (Bosch *et al.*, 1998; Sorensen *et al.*, 2013).

Cover crop residue decomposition and mineralization after incorporation into or onto the soil provides extra-C and -N to soil microorganisms (Maris *et al.*, 2021), promoting N_2O emissions and affecting the yield of the following crop (Fiorini *et al.*, 2020; Martínez-García *et al.*, 2021). Since soil water content and wetting–drying cycles are strong regulators of fresh residue decomposition (Schmidt *et al.*, 2016), contrasting irrigation systems may determine different cover crop decomposition rates, resulting in differences in soil C and N availability. This could be one of the main mechanisms by which irrigation practices regulates N-use efficiency in crop rotations including cover crops. However, no prior studies have examined how different irrigation methods control cover crop decomposition rate and the associated impacts on N_2O emissions and crop yield.

In a two-year field study, we investigated the effect of three irrigation systems (subsurface drip irrigation with a narrow dripline spacing [SDI70] vs. subsurface drip irrigation with a wide dripline spacing [SDI140], vs sprinkler irrigation [SPR]) on yield, N_2O emissions and N-use efficiency of maize and soybean. We hypothesized that: (1) micro-irrigation techniques (SDIs) combined with split fertigation increase crop yield and NUE while reducing N_2O emissions compared with SPR; (2) the positive effect of SDI on crop yield, NUE and N_2O emissions is stronger on maize than on soybean; (3) among SDI systems, a narrow dripline spacing (70 cm) increases crop yield and NUE while reducing N_2O emissions compared to a wide dripline spacing (140 cm); and (4) SDI systems reduce litter decomposition rate and, therefore, curb N_2O emissions.

2. Materials and methods

2.1 Site description

We set a two-year field experiment at CERZOO experimental research station in Piacenza (45°00′18.0″N, 9°42′12.7″E; 68 m above sea level), Po Valley, Northern Italy. The soil is a fine, mixed, mesic Udertic Haplustalfs (Soil Survey Staff, 2014), with a silty clay texture. The physico-chemical characteristics of the soil in the top 0-30 cm layer were: sand 123 g kg^{-1}; silt 466 g kg^{-1}; clay 412 g kg^{-1}; pH H_2O 7.6; organic matter concentration 33 g kg^{-1}; bulk density 1.30 g cm^{-3}; soil total N 1.9 g kg^{-1}; available P (Olsen) 43 mg kg^{-1}; exchangeable K (NH_4^+ Ac) 292 mg kg^{-1}; and cation exchange capacity 32 $cmol^+$ kg^{-1}. The site is characterized by a temperate climate (Cfa as Köppen classification), with average annual temperature of 13.2 °C and annual rainfall of 837 mm (average of 2000-2020 period). Average temperature and rainfall during maize and soybean growing season (average of 2000-2020 period) are 21.5 °C and 300 mm, respectively. Meteorological data during the experiment were collected from an automated meteorological station placed near the experimental field. Growing season cumulative rainfall was calculated as the sum of daily cumulative rainfall between sowing and harvest of main crops.

2.2 Experimental design, treatments and crop management

A subsurface drip irrigation (SDI) plant was designed and established in April 2014. In detail, two SDI sectors, each of 13400 m^2, were arranged within the selected experimental field. With GPS positioning drip pipes and laterals were buried to 45 cm below the soil surface in all sectors, while the inter-row spacing was 70 cm in one sector and 140 cm in the other sector, thus defining two different structural set-ups of the plant as two SDI experimental levels: (i) subsurface drip irrigation with inter-row of 70 cm (SDI70), and (ii) subsurface drip irrigation with inter-row of 140 cm (SDI140). Since sprinkler irrigation (SPR) is the most common irrigation systems of the area (Po Valley, right side of the Po river), an additional sector of the field (alongside with the two sectors with SDI) were sprinkler irrigated as control, keeping a 3 m buffer zone between SDI and SPR sectors. Irrigation in the SPR system was carried out with a hose reel system, which consists of a single portable sprinkler head spraying water in a circular pattern. The irrigation flow rate was 3200 L min^{-1} and the lateral length was 400 m.

Prior to set up the SDI plant, the soil was managed with conventional agriculture practices (i.e. moldboard plowing at 40-cm depth and rotary harrowing at 15-20-cm depth, no cover crops, no crop residues left). Starting from April 2014 right after SDI setup, conservation agriculture has been adopted (i.e. no-till plus cover crops and residue management). From May 2014 to October 2018, the crop sequence was a maize-soybean crop rotation. The present field experiment started in December 2018, four years after the conversion to conservation agriculture, so excluding any interactions due to possible effects of the transition (Fageria *et al.*, 2007; Derpsch *et al.*, 2014; Pittelkow *et al.*, 2015) and maintained until December 2020. Throughout the experiment, soybean (*Glycine max* [L.] Merr.; cv. Xonia) and maize (*Zea mays* L.; hy. LG 30.597) were planted as main crops for all three irrigation systems, both simultaneously present on the field in each year by splitting the three main sectors (SDI70, SDI140 and SPR) into six sub-sectors of 6700 m^2 each. Therefore, the following six treatments were established: SDI70 with maize (SDI70-M), SDI70 with soybean (SDI70-S), SDI140 with maize (SDI140-M), SDI140 with soybean (SDI140-S), SPR with maize (SPR-M) and SPR with soybean (SPR-S). The experimental field and treatment design are displayed in Figure S1. The maize-soybean sequence was kept in all sectors, so that maize was planted in 2020 in sectors where soybean was planted in 2019, and *viceversa*. In 2019, maize and soybean were planted on June 6th because of high cumulative rainfall during the April-May period (269 mm; Figure S2), which resulted in excessive soil moisture content for planting, compared to the same period in 2020 (168 mm; Figure S2), when both crops were planted earlier (on April 23rd). In both years, crop rows were aligned on top of driplines thanks to GPS assisted planter. Right after harvesting the main crop (on 12th October in 2019 and on 24th September in 2020), a cover crop mixture including 26% (on weigh) rye (*Secale cereale* L.), 16% common oat (*Avena sativa* L.), 12% black oat (*Avena strigosa* Schreb.), 16% hungarian vetch (*Vicia pannonica* Crantz.), 20% common vetch (*Vicia sativa* L.), 3% crimson clover (*Trifolium incarnatum* L.), 2% berseem clover (*Trifolium alexandrinum* L.), and 5% tillage radish (*Raphanus sativus* L. subsp. *longipinnatus*) was sown each year at rate of 60 kg ha^{-1}. Approximately two weeks before sowing the following main crop, cover crops were chemically terminated by spraying Glyphosate [N-(phosphonomethyl) glycine] at the rate of 3 L ha^{-1} and residues were left onto the soil surface without mowing. Maize and soybean were sod-seeded after cover crop termination.

The experimental design consisted of three factors: (i) the irrigation system as the first factor, with three levels (SDI70, SDI140 and SPR); (ii) the crop type as the second factor with two levels (Soybean, S and Maize, M), which was nested within the irrigation factor; and (iii) the experimental year as the third factor, with two levels (2019 and 2020). As a result, the six irrigation system × crop type treatments were present simultaneously in both years. The number of pseudo-replicates - within each of the six sub-sectors - was four.

Irrigation water requirements during the maize and soybean cropping cycles were calculated for each treatment as follows: ETc = Kc × ET0, where ETc is the theoretical crop evapotranspiration, ET0 is the reference evapotranspiration calculated by the FAO Penman-Monteith formula, while Kc is the single crop coefficient calculated according to the crop stages (Kc ini; Kc mid; and Kc end) (Allen *et al.*, 1998). Partial soil wetting of the SDI system was taken into account multiplying Kc ini by the fraction of the soil surface wetted (Allen *et al.*, 1998) (estimated as a 5%), which was based on visual estimation of surface soil wetting during irrigations (Hunsaker and Bronson, 2021). As a result, irrigations were performed every 10-14 days on SPR and every 3-4 days on SDI, on average. Irrigation-water use efficiency [iWUE (kg m^{-3})] was calculated as the ratio between grain yield (kg ha^{-1}) and supplied irrigation water (m^3 ha^{-1}). N-fertilization was only used in maize (both years). N-fertilizers were split and applied to maize in SDI treatments every 7-10 days as ammonium sulphate [$(NH_4)_2SO_4$] through the drip system with repeated applications of 40 kg N ha^{-1} each, totaling 280 kg N ha^{-1}. In maize SPR treatment, N-fertilizers were supplied as granular urea (CH_4N_2O), with two applications (140 kg N ha^{-1} each) at V2-V3 and at V8-V9 growth stages in both years, using a tractor with a fertilizer spreader. Urea incorporation into the soil under SPR treatments was promoted by a subsequent irrigation. The N-fertilizer rate was computed according to the estimated (predicted) N-balance, considering crop, soil, and climate variables (Grignani *et al.*, 2007).In detail, the N rate to be supplied with fertilizer was calculated as the difference between the estimated crop N-uptake (considering reasonable target yields for the area) and the estimated available N in the soil. According to soil physical and chemical properties, estimated available N was calculated as follows (Grignani *et al.*, 2003):

$$Na = Nm - Nl \pm Nr + Ns - Nid \tag{1}$$

where (i) *Nm* is the estimated mineralized N according to organic matter and total N concentration, soil texture, soil C:N ratio and bulk density; (ii) *Nl* is the estimated rate of N leached, as a function of rain and irrigation rates; (iii) *Nr* is the residual N, estimated according to previous crops and cover crops; (iv) *Ns* is supplemental N from previous organic amendments (if any), atmospheric deposition and irrigation water; and (v) *Nid* is the immobilized and/or dispersed N. Goodness of estimation was then verified by confirming the minor changes in soil total N and available pools at the end of the experiment.

2.3 Yield measurements

Yield components of maize and soybean crops were assessed by manually harvesting four areas of 10 m^2 per single sub-sector (8 and 50 plant per m^2 for maize and soybean, respectively). Plants were weighed and separated into grain and stover. A 100 g sub-sample of each grain and stover sample was oven-dried at 65 °C until constant weight to measure dry matter content. Soybean stover was measured at harvest, also collecting fallen leaves. Harvest index (HI) of maize and soybean was calculated as the ratio between grain yield and total biomass at harvest on a dry matter basis. Grain and total N-uptake were calculated by multiplying grain yield and grain yield + stover biomass by their N-concentrations, respectively. N-concentrations of grain and stover were determined by the Dumas combustion method with an elemental analyzer varioMax C:N (VarioMax C:NS, Elementar, Germany).

Regarding cover crops, four areas of 3 m^2 each were randomly chosen within each sub-sector by manually harvesting plants and weighed to assess total aboveground biomass. Sub-samples were collected to calculate dry matter content and C and N concentration as described above for main crops.

2.4 N-fertilization efficiency measurements

The three following N-efficiency parameters were calculated for each treatment according to López-Bellido and López-Bellido (2001): (i) N-use efficiency (NUE; kg kg^{-1}) as the ratio of grain yield to N supply, where N supply is the sum of soil nitrate (NO_3^-) at sowing, mineralized N and N-fertilizer; (ii) N harvest index (NHI; %) as the ratio of N in grain to N in total plant biomass; and (iii) N-utilization efficiency (NU_tE; kg kg^{-1}) as the ratio of grain yield to total plant N-uptake. The actual mineralized N was calculated at the end of the experiment according to Feichtinger *et al.* (2004) as follows:

$$Nm = Nd + Np - Nf \tag{2}$$

where (i) Nm is the net-N-mineralisation (kg N ha^{-1}); (ii) Nd is the difference in inorganic N in the soil (0–30 cm) between autumn and spring, (kg N ha^{-1}); (iii) Np is the N uptake by plants (kg N ha^{-1}); and (iv) *Nf* is the inorganic N fertilisation through mineral fertiliser (kg N ha^{-1}).

2.5 Nitrous oxide sampling and flux estimates

The close chamber method (Smith *et al.*, 1995; Moretti *et al.*, 2020) was used to assess N_2O direct emissions from soils from December 2018 to December 2020. Cylindrical static chambers (40 cm diameter and 25 cm high) were made of polyvinyl chloride (PVC) with a light color to reduce the impact of direct radiating heat during samplings. The chambers (four per treatment) were inserted into the soil by fitting them into stainless steel rings, which were positioned 10 cm into the soil prior to the beginning of the experiment. Rings were temporarily removed exclusively for specific operations (i.e. planting, fertilizing and harvesting) in order to avoid the effect of soil disturbance on N-fluxes. Chambers were centered at 17.5 cm and 35 cm from driplines (and rows) for SDI70 and SDI140 respectively, as a way to manage different dripline spacing (Figure S1). In SPR sectors, chambers were centered in inter-row (Figure S1). A battery-operated fan was installed inside each chamber to maintain air mixing. Gas sampling took place once per month during winter periods (due to the low soil temperatures and absence of intense freeze-thaw cycles) up to twice/three times per week following N-fertilizer applications. The total number of measurements was 40 (20 per year). As described by Maris *et al.* (2015 and 2018) sampling was carried out in the morning between 09:00 and 12:00 hours to reduce diurnal variation in flux patterns. Alongside N_2O sampling, the temperature outside and inside the chambers was measured with digital thermometers. Six ambient air samples were taken at the moment of chamber closure (at 0 min) and then headspace air samples were taken at 15 and 30 min after enclosure of chambers. A 100 mL syringe was used to collect 60 mL air samples; a volume of 30 mL was discarded to purge the syringe and the remaining gas was transferred to 12 mL pre-evacuated LabcoExetainer® glass vials sealed with butyl rubber stoppers. Subsequently, air samples were analyzed by gas chromatography (Agilent 7890A with a Gerstel Maestro MPS2 autosampler) equipped with an electron capture detector for N_2O quantification. The linear increase of N_2O concentration (after temperature corrections) within the chamber headspace was used to calculate daily fluxes when linearity was verified ($R^2 > 0.9$). Emission rates were estimated as the slope of the linear regression between concentration and time and from the ratio between chamber volume and soil surface area (MacKenzie *et al.*, 1998). Annual cumulative N-N_2O emissions were calculated by linear interpolation of the whole annual sets of fluxes, while growing season cumulative N-N_2O emissions were calculated for each experimental year by linear interpolation of fluxes measured from sowing to harvest.

2.6 Soil properties

Four soil samples were collected to determine mineral N-content from each sub-sector once per month in winter periods, and up to twice/three times per week after N-fertilizer applications. Each soil sample consisted of 3 soil sub-samples taken at 0, 17.5 and 35 cm from the dripline in SDI70 treatments, at 0, 35 and 70 cm from the dripline in SDI140 treatments, and at 0, 35 and 70 cm from the crop row in SPR treatments, as a means to account for the different N-fertilizer spatial patterns (Figure S1). The soil cores were taken at 30 cm depth and then divided into two layers: 0-10 and 10-30 cm. Finally, four composite soil samples were obtained per each depth, sampling date, and sub-sector. Soil samples were immediately transported to the laboratory for NO_3^-, ammonium (NH_4^+) and water content analyses. The soil NO_3^- and NH_4^+ concentrations were analysed using 5 g of homogeneously mixed soil extracted with 20 mL of K_2SO_4 (0.5 M) and pipetted into 96-well quartz microplates. Nitrate-N and NH_4^+-N were then determined with dual-wavelength UV spectroscopy (275, 220 nm) on acidified (HCl 1 M) samples. Gravimetric water content (GWC) in the 0-10 cm soil layer was measured at each gas sampling by oven drying soil samples at 105 °C for 24 h. The cylinder method (Gómez-Paccard *et al.*, 2015) was used to assess soil bulk density at 0-10 cm depth. Volumetric water content (VWC) at 0-10 cm depth was calculated by multiplying GWC and soil bulk density, while soil porosity was determined assuming mineral soil particle density of 2.65 g cm^{-3} (Porta Casanellas and López-Acevedo Reguerín, 2008). Field capacity was calculated as described by Saxton and Rawls (2006). Water-filled pore space (WFPS) was calculated as the ratio of VWC and soil porosity (Danielson and Sutherland, 1986). The average growing season soil WFPS, NO_3^- and NH_4^+ concentrations were calculated as weighted means of data measured from planting to harvest.

2.7 Litter and litter-N decay rate *k*

In both years during maize and soybean seasons, nylon litter-bags (40 × 30 cm; 1 mm size) were filled with 50 g of cover crop residues previously dried at 65 °C until constant weight (Bocock and Gilbert, 1957). These litter-bags were randomly placed on the soil surface after cover crops termination. For each irrigation sub-sector, four litter-bags were collected at each sampling time (at 8, 18, 30, 46, 65, 90, 121 and 156 days after positioning). Then, litter-bags were dried at 65 °C and weighed for estimating mass decay rate. N-concentration was determined by the Dumas combustion method described above and corrected considering ash-content as described by Christensen (1985). Litter-DM and litter-N decay rate k (day^{-1}) were calculated according to Olson (1963) as follows:

$ln(Mt\ /\ M0)\ =\ -k\ t$ for litter-DM decay rate k, (3)

$ln(Nt\ /\ N0)\ =\ -k\ t$ for litter-N decay rate k, (4)

where $Mt\ /\ M0$ is the fraction of initial litter mass remaining at time t, $Nt\ /\ N0$ is the fraction of initial litter-N remaining at time t, t is the elapsed time (d), and k is the decay constant (d^{-1}). Average growing season litter-DM and litter-N decay k were calculated as weighted means of data measured from sowing to harvest of main crops.

2.8 Statistical analyses

A linear mixed model was applied to study the effect of the irrigation treatment, crop and year on (i) maize and soybean grain yield and total biomass, (ii) N-uptake and N-efficiency parameters, (iii) cumulative N_2O emissions, and (iv) growing season average soil NO_3^- and NH_4^+ content. The subplot within sub-sectors was considered as a random factor. We used linear mixed effects models to account for the lack of independence among the individual units of observation. The measured variables were checked for normality using the Shapiro-Wilk test and for homogeneity of variances with the Levene’s test.

A repeated measures ANOVA was conducted, separately for the two experimental years, on litter and litter-N decay k with irrigation, crop and time as fixed factors and replicate as random effect. When the ANOVA assumptions were violated, data were log transformed prior to analysis and back-transformed after the post hoc test. Tukey’s honestly significant difference (HSD) was used as post hoc to test significant differences among treatments with a p-value of 0.05 as threshold. The correlation analysis was performed to assess the relationship between all variables measured or calculated in the experiment, using the non-parametric Spearman rank coefficient (ρ). A p-value of 0.05 was considered significant for the test. We used R 4.0.3. (R Core Team, 2020) with nlme (Pinheiro *et al.*, 2013), multcomp (Hothorn *et al.*, 2007), and factoextra (Kassambara and

Mundt, 2020) packages for the linear mixed effect models, HSD tests and Spearman's rank correlations, respectively.

3. Results

3.1 Environmental conditions, water parameters and soil mineral N pools

Average daily air temperature during the two-year period ranged from 1.2 to 25.0 °C, while annual rainfall was 1020 mm in 2019 and 949 mm in 2020 (Figure S2).

	Treatment	Cumulative rainfall (mm)	Total irrigation (mm)	Rainfall + irrigation (mm)	Cumulative ETc (mm)
2019	SDI70-M	194	243	437	429
	SDI70-S		182	376	371
	SDI140-M		243	437	429
	SDI140-S		182	376	371
	SPR-M		295	489	481
	SPR-S		231	425	418
2020	SDI70-M	347	163	510	503
	SDI70-S		101	448	439
	SDI140-M		163	510	503
	SDI140-S		101	448	439
	SPR-M		200	547	540
	SPR-S		132	479	474

Table 1. Growing season water parameters for each treatment in 2019 and in 2020 (subsurface drip irrigation with dripline distance of 70 cm on maize [SDI70-M], subsurface drip irrigation with dripline distance of 70 cm on soybean [SDI70-S], subsurface drip irrigation with dripline distance of 140 cm on maize [SDI140-M], subsurface drip irrigation with dripline distance of 140 cm on soybean [SDI140-S], sprinkler irrigation on maize [SPR-M] and sprinkler irrigation on soybean [SPR-S]).

Despite the similar annual rainfall, the two years had a very different rainfall pattern during the maize and soybean growing season period. In detail, growing season cumulative rainfall was 194 mm in 2019, while the corresponding value in 2020 was 347 mm (+79%) (Figure S2; Table 1). Cumulative growing season ETc under maize SDI treatments was 429 mm in 2019 and 503 mm in 2020 (Table 1). Water applied to maize via irrigation was 243 mm in 2019 and 163 mm in 2020 (-33%) (Table 1). Therefore, total water applied to maize under SDI treatments was 437 mm in 2019 and 510 mm in 2020

(Table 1). Further details about irrigation rates and ETc for SPR treatments and soybean are reported in (Table 1).

Water-filled pore space was generally higher under SPR than under SDI (Figure S3). Specifically, average WFPS for the growing season was 54% in 2019 and 58% in 2020 under SDI, while it was 61% in 2019 and 64% in 2020 under SPR (Figure S3). Among SDI treatments, the narrow dripline distance slightly reduced average growing season WFPS from 55% to 54% in 2019 and from 60% to 57% in 2020 compared with SDI140 (Figure S3). However, the effect of dripline distance on average growing season WFPS was more pronounced on maize in 2019 (Figure S3): SDI70-M reduced WFPS by 7% compared with SDI140-M. Overall, average growing season WFPS was lower in 2019 (57%) than in 2020 (61%).

Concentrations of NO_3^- and NH_4^+ during the growing season in the 0-10 and 10-30 cm soil layers were affected by the three-factor interaction (Table 2). Nitrate concentration was significantly higher under SDI140-M than under SDI70-M in the 0-10 cm soil layer in 2019, while both SPR-M and SDI140-M increased NO_3^- concentration compared with SDI70-M in the 10-30 cm soil layer during the same year. No difference between treatments was found in 2020 (Figure 1a).

NO_3^- concentration in the 0-10 cm soil layer was higher under SDI-S treatments than under SPR-S in 2019, whereas only SDI140-S increased soil NO_3^- content compared with SPR in 2020 (Figure 1b). SPR-S reduced NO_3^- concentration in the 10-30 cm soil layer compared with SDI140-S in 2019 (Figure 1b).

Ammonium concentration was often higher for SPR-M in 2019 (+67% and +32% in the 0-10 cm layer compared with SDI70-M and SDI140-M, respectively) (Figure 1c). SDI70-M significantly reduced average soil NH_4^+ concentration in both soil layers compared with other treatments in 2019 (Figure 1c). In 2020, NH_4^+ soil concentration was significantly higher under SPR-M than under SDI treatments only in the 0-10 cm layer (Figure 1c). No differences were found between soybean treatments regarding NH_4^+ concentration in the 0-10 cm soil layer for both years, however SDI140-S increased NH_4^+ content in the 10-30 cm soil layer in 2020 (Figure 1d).

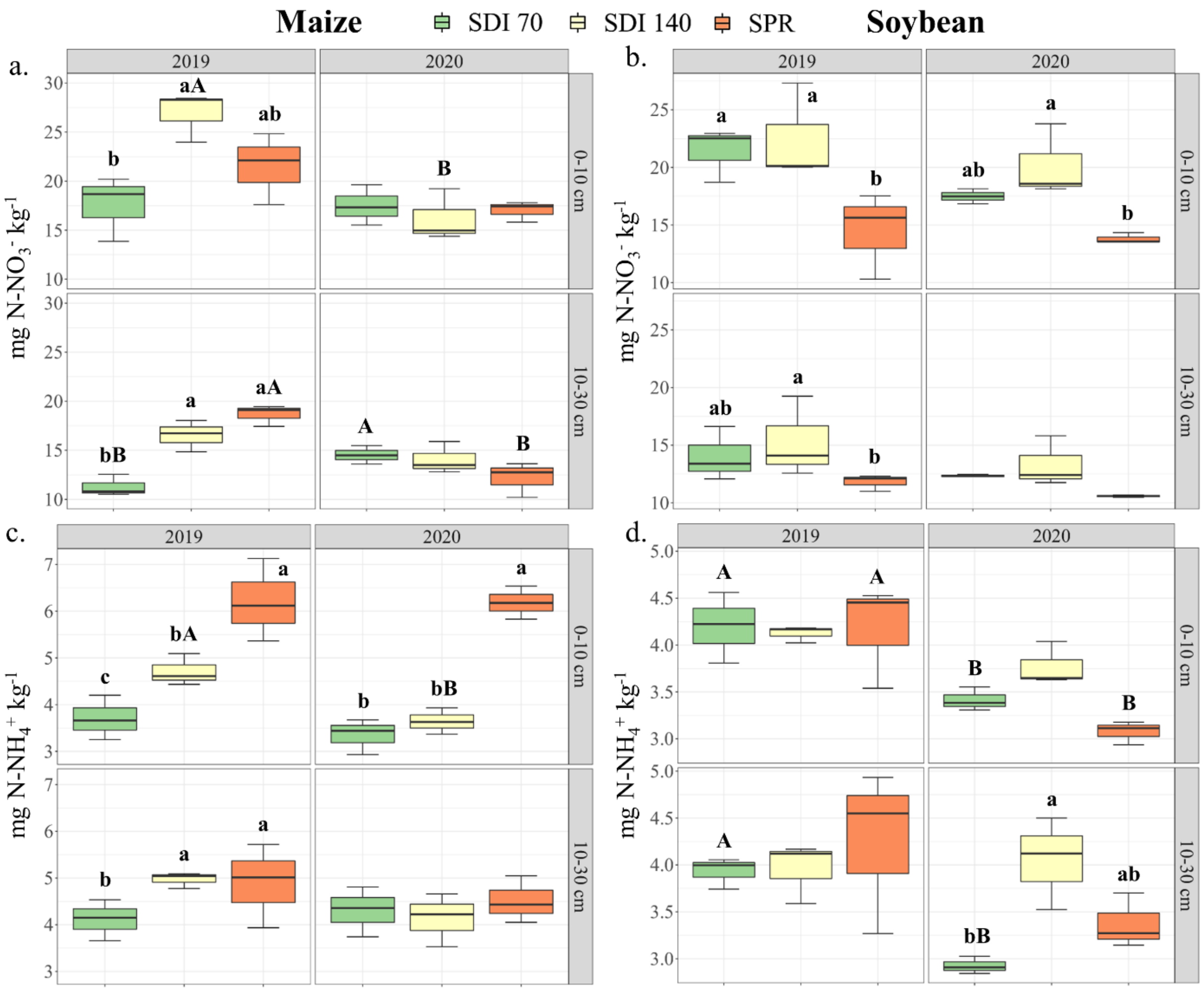

Figure 1. Box plots of N-NO_3^- concentration on maize (a) and soybean (b); N-NH_4^+ concentration on maize (c) and soybean (d) in the 0-10 cm and 10-30 cm soil layers as affected by year (2019 and 2020) and irrigation system (subsurface drip irrigation with dripline distance of 70 cm [SDI70], subsurface drip irrigation with dripline distance of 140 cm [SDI140] and sprinkler irrigation [SPR]). The bottom and top of each box represent the lower and upper quartiles respectively, the line inside each box shows the median and whiskers indicate minimal and maximum observations. Capital letters indicate differences among years within the same irrigation system; lowercase letters indicate differences among irrigation systems within the same year. Please note the scale differences in the Y-axis between crops.

3.2 Yield components and efficiency parameters

All the yield and efficiency parameters (i.e. NUE, NHI, NUtE, and iWUE) were affected by the three-factor interaction (Table 2) and are reported in Table 3. SDI generally increased maize grain yield compared with sprinkler irrigation in 2019 (+31%). In addition, SDI70 had higher NUE, NHI and NUtE (while SDI140 had only higher NUtE) than SPR for maize in 2019. Maize HI was reduced by SPR compared with SDI treatments in 2019. No differences were found between SDI70-M and SPR-M during 2020 in terms of N-efficiency parameters, while SDI140-M had lower NUE and NUtE than SPR-M and SDI70-M in 2020. SPR-M and SDI70-M outyielded SDI140-M in 2020 (+25% and +16% respectively). Both SDI70-M and SPR-M significantly increased grain yield in 2020 compared with 2019. Moreover, SPR increased HI of both soybean and maize in 2020 compared with 2019. Conversely, soybean grain yield and N-efficiency parameters were not affected by the irrigation treatments or years. Total biomass was lower for SDI140-M than for SDI70-M and SPR-M in 2020, while no difference between treatments occurred in 2019. Similar to grain yield, total biomass under SDI70-M and SPR-M was higher in 2020 than in 2019. HI was higher in 2020 than in 2019 under SPR-M and SPR-S.

Grain and total biomass N-uptake were not affected by the treatments, but it was generally higher in 2020 than in 2019.

Irrigation-water use efficiency of maize was higher under SDI treatments than under SPR in 2019, whereas both SPR-M and SDI140-M had lower iWUE compared with SDI70-M in 2020. No differences were found between soybean treatments in terms of iWUE in both 2019 and 2020.

Source of variation	Grain Dry Yield	Total Dry Biomass	HI	Grain N-uptake	Total Biomass N-uptake	NUE	NHI	NUtE	iWUE	Annual cumulative N_2O emission	Growing season cumulative N_2O emission	0-10 cm growing season NO_3^-	10-30 cm growing season NO_3^-	0-10 cm growing season NH_4^+	10-30 cm growing season NH_4^+
								p-value							
Year (Y)	**< 0.001**	**0.005**	**< 0.001**	**< 0.001**	**< 0.001**	0.579	**0.022**	0.211	**< 0.001**	**0.030**	**0.011**	**< 0.001**	**0.005**	**< 0.001**	**< 0.001**
Crop (C)	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**0.004**	**0.055**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	0.221	**0.008**	**< 0.001**	**< 0.001**
Irrigation (I)	0.175	0.135	**0.002**	**0.009**	**< 0.001**	**< 0.001**	**0.002**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**0.001**	**0.040**	**< 0.001**	0.004
Y×C	**< 0.001**	**< 0.001**	**0.041**	**0.020**	**0.019**	**< 0.001**	**0.019**	**0.005**	**< 0.001**	**0.003**	**0.015**	0.138	0.810	**< 0.001**	0.191
Y×I	**0.002**	0.211	**< 0.001**	0.803	0.175	**< 0.001**	**< 0.001**	**< 0.001**	0.176	0.473	0.567	0.111	**0.010**	0.850	0.670
C×I	**< 0.001**	0.109	**< 0.001**	0.157	0.492	**0.002**	**0.002**	**< 0.001**	**< 0.001**	0.234	0.125	**0.014**	**0.011**	0.290	0.436
Y×C×I	**< 0.001**	**0.036**	**0.006**	**0.014**	**0.003**	**0.007**	**0.038**	**< 0.001**	**< 0.001**	0.347	0.156	**0.037**	**0.005**	**0.020**	**0.002**

Table 2. Analysis of variance of yield components, N-fertilizer efficiency parameters, irrigation-water use efficiency, N_2O emissions and soil N pools as affected by year (Y), irrigation system (I), crop (C) and the interactions between factors.

Source of variation			Grain Dry Yield (Mg ha^{-1})		Total Dry Biomass (Mg ha^{-1})		HI (%)		Grain N-uptake (kg ha^{-1})		Total Biomass N-uptake (kg ha^{-1})		NUE (kg kg^{-1})		NHI (%)		NUtE (kg kg^{-1})		iWUE (kg m^{-3})	
Year x Irrigation x Crop																				
2019	Maize	SDI-70	11.9	a B	20.8	a B	57	a A	151	a B	211	a B	31.2	a A	72	a A	56.4	a A	4.99	a B
		SDI-140	11.9	a A	22.0	a A	54	a A	153	a A	239	a B	24.9	b A	64	ab A	49.7	b A	4.88	a B
		SPR	9.1	b B	22.3	a B	41	b B	146	a B	264	a A	23.3	b B	55	b A	34.7	c B	3.09	b B
	Soybean	SDI-70	3.3	a A	10.6	a A	31	a A	213	a A	253	a A	33.9	a A	85	a A	13.0	a A	1.64	a B
		SDI-140	3.6	a A	11.3	a A	32	a A	230	a A	269	a B	22.1	b A	86	a A	13.4	a A	1.79	a B
		SPR	3.8	a A	12.1	a A	31	a B	239	a A	292	a A	29.4	a A	82	a B	12.9	a A	1.52	a B
2020	Maize	SDI-70	14.6	a A	26.3	a A	55	a A	197	a A	306	a A	31.9	a A	65	a B	47.9	a B	8.95	a A
		SDI-140	11.7	b A	22.6	b A	52	a A	178	a A	284	a A	25.6	b A	63	a A	41.2	b B	7.19	b A
		SPR	13.6	a A	26.4	a A	52	a A	189	a A	292	a A	32.5	a A	65	a A	47.0	a A	6.82	b A
	Soybean	SDI-70	3.3	a A	10.0	a A	33	a A	219	a A	256	b A	21.0	b A	86	a A	12.9	a A	2.73	a A
		SDI-140	4.4	a A	11.5	a A	38	a A	282	a A	321	a A	23.9	b A	89	a A	13.8	a A	3.66	a A
		SPR	4.4	a A	10.7	a A	41	a A	279	a A	320	a A	32.4	a A	88	a A	13.8	a A	2.94	a A

Table 3. Grain dry yield (Mg ha^{-1}), total dry biomass (Mg ha^{-1}), harvest index (%), grain N-uptake (kg ha^{-1}), total biomass N-uptake (kg ha^{-1}), nitrogen use efficiency (NUE; kg kg^{-1}), nitrogen harvest index (NHI; %), nitrogen utilization efficiency (NUtE; kg kg^{-1}) and irrigation-water use efficiency (iWUE; kg m^{-3}) as affected by the interaction between year, (2019 and 2020) irrigation system (subsurface drip irrigation with dripline distance of 70 cm [SDI70], subsurface drip irrigation with dripline distance of 140 cm [SDI140] and sprinkler irrigation [SPR]) and crop (maize and soybean). Capital letters indicate differences among years within the same irrigation system and crop; lowercase letters indicate differences among irrigation system within the same year and crop.

3.3 N_2O fluxes and cumulative emissions

The daily average fluxes ranged from 0.00 to 0.52 kg N-N_2O ha^{-1} d^{-1} during the two experimental years, and the highest value was measured in 2020 on SDI140-M (Figure 2c). In 2019, N_2O emissions remained low until cover crops were terminated and irrigations (as well as fertilizations in maize) were carried out in late June. Then, a major emission peak occurred following the two urea applications under SPR-M and three emission peaks were observed after fertigation events under SDI70-M and SDI140-M (Figure 2a). Several emission peaks were observed after cover crops termination and irrigations in 2019 under soybean treatments, especially in SDI140-S and SPR-S (Figure 2b). In 2020, N_2O emission peaks were amplified (exclusively for maize) compared with previous year and occurred earlier (by the end of May), as a consequence of earlier sowing, fertilizations, and irrigations dates (Figures 2c and 2d). Two major peaks were observed in all maize treatments following urea applications or fertigations in 2020 (Figure 2c). N_2O emissions increased after cover crops termination and irrigations under soybean treatments in 2020 (Figure 2d).

Annual and growing season cumulative N_2O emissions were affected by irrigation and by the interaction between crop and year (Table 2). Specifically, SDI70 had lower N_2O emissions than SDI140 and SPR in both annual (7.2 kg ha^{-1}; -44% and -36% respectively) and growing season (5.1 kg ha^{-1}; -44% and -39% respectively) cumulative emissions (Table 4). During 2020, N_2O emissions were significantly higher than those in 2019 on maize, whereas no differences between years were found for soybean (Table 4).

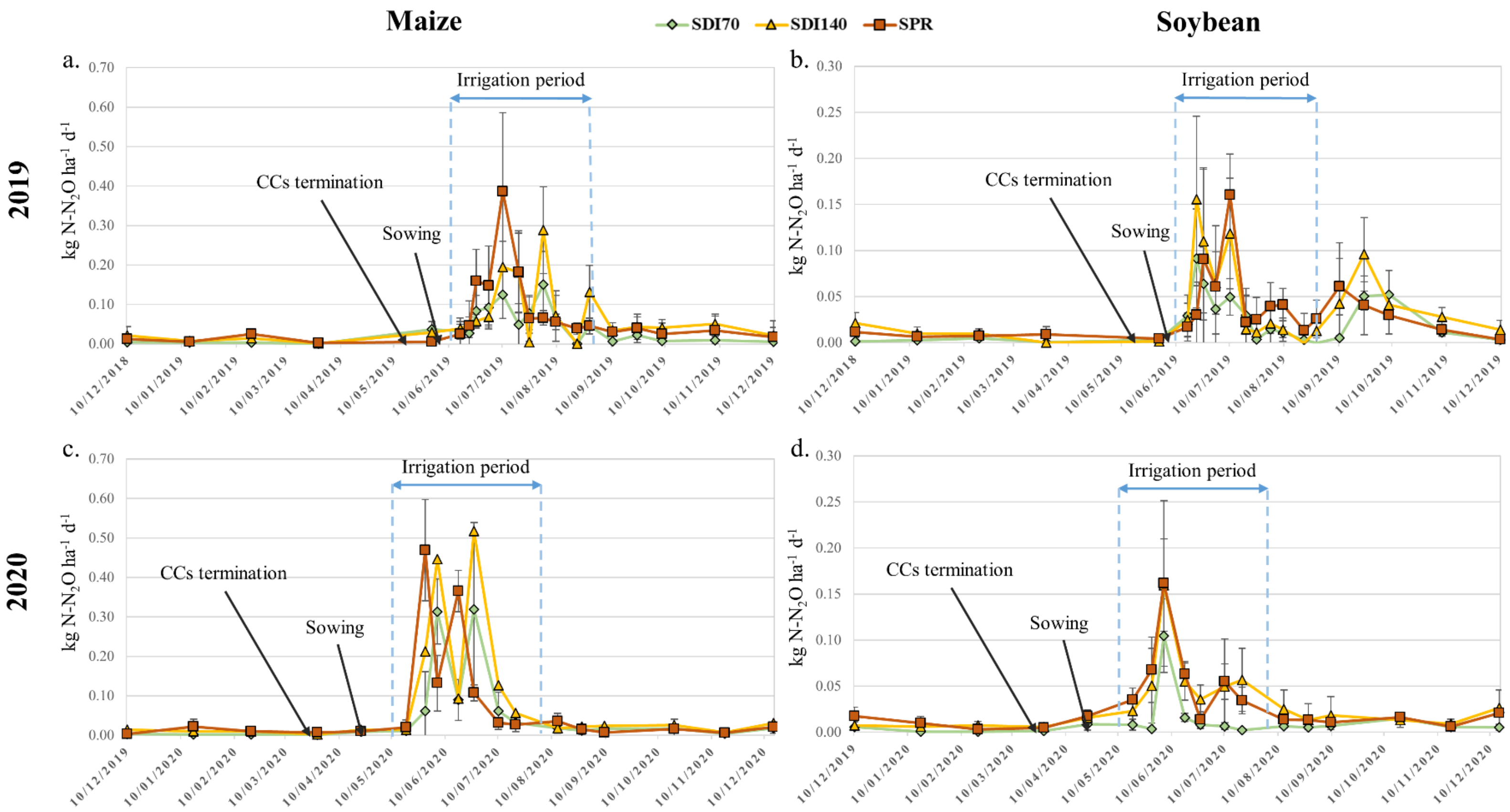

Figure 2. Daily fluxes of N_2O on maize and soybean during the experiment (from December 2018 to December 2020) as affected by irrigation systems (subsurface drip irrigation with dripline distance of 70 cm [SDI70], subsurface drip irrigation with dripline distance of 140 cm [SDI140] and sprinkler irrigation [SPR]). Error bars in the figure represent standard deviation. Please note the scale differences in the Y-axis between crops.

Source of variation		Annual cumulative N_2O emissions ($kg\ N_2O\ ha^{-1}\ y^{-1}$)		Growing season cumulative N_2O emissions ($kg\ N_2O\ ha^{-1}$)	
Irrigation					
SDI-70		7.2	b	5.1	b
SDI-140		12.9	a	9.1	a
SPR		11.2	a	8.3	a
Crop x Year					
Maize	2019	12.3	b	8.3	b
	2020	15.9	a	13.2	a
Soybean	2019	7.1	a	4.0	a
	2020	6.4	a	4.4	a

Table 4. Annual and growing season cumulative N_2O emissions as affected by irrigation system (subsurface drip irrigation with dripline distance of 70 cm [SDI70], subsurface drip irrigation with dripline distance of 140 cm [SDI140] and sprinkler irrigation [SPR]) and the interaction between crop (maize and soybean) and year (2019 and 2020).

3.4 Litter and litter-N decay rates

Litter and litter-N decay rates were significantly affected by the interaction between time, crop and irrigation (Table 5). Maize and soybean litter decay rates were generally lower in 2019 than in 2020. For both years and crop types, SPR increased litter and litter-N decay compared to SDI (Figure S4).

Source of variation	Litter decay *k* 2019 (d^{-1})	Litter decay *k* 2020 (d^{-1})	Litter-N decay *k* 2019 (d^{-1})	Litter-N decay *k* 2020 (d^{-1})
		p-value		
Time (T)	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
Irrigation (I)	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
Crop (C)	**0.001**	**< 0.001**	**0.020**	**< 0.001**
T×I	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**
T×C	**< 0.001**	**< 0.001**	**< 0.001**	**0.001**
C×I	**< 0.001**	**< 0.001**	**< 0.001**	**0.003**
T×C×I	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**

Table 5. Analysis of variance of litter decay rate *k* and litter-N decay rate *k* in 2019 and 2020 as affected by time (T), irrigation system (I), crop (C) and interactions between factors.

3.5 Relationships between variables

Grain yield, total biomass, grain N-uptake and total biomass N-uptake were positively correlated between them for both maize and soybean (Figure S5a-b). Maize N-efficiency parameters were positively correlated between each other (Figure S5a), whereas only NUtE was positively correlated with NHI on soybean (Figure S5b). Among maize N-efficiency parameters, NHI and NUtE were negatively correlated with WFPS (Figure S5a). Maize annual cumulative N_2O emissions were positively correlated with litter decay *k* and negatively correlated with NUtE, while growing season cumulative N_2O emissions were positively correlated with grain and total biomass N-uptake, cumulative rainfall and litter decay *k* (Figure S5a).

Soybean annual and growing season cumulative N_2O emissions had positive correlations with grain yield, total biomass, N-uptake in grain and biomass, and litter-N decay *k* (Figure S5b).

4. Discussion

4.1 Yield, N-efficiency and iWUE responses of maize and soybean to irrigation and N-fertilization method

The generally higher maize yield and NUE of SDI in 2019 compared to SPR suggests a potential higher capacity for this irrigation method to improve N exploitation and relocation in maize grain. The increased maize yield, NUE and iWUE of SDI observed in 2019 was likely because of: (i) reduced ETc under SDI than under SPR, as supported by the WFPS results, and (ii) more efficient water and N-fertilizer distribution, which are supplied together and directly close to the root zone in SDI, at 45-cm soil depth in our case. On the other hand, with SPR irrigation, water is applied on the top of the soil surface every 10-14 days in relatively high amounts, promoting high evaporation losses. Moreover, under SPR fertilization is performed with two applications of N-fertilizer (140 kg N ha^{-1} each), thus possibly leading to a mismatch between soil N availability and plant N demand. Hence, both these factors led to reduced irrigation- and N-use efficiency (Li *et al.*, 2018; Sandhu *et al.*, 2019). This is supported by the higher soil moisture under SPR during maize in 2019, and by the negative relationship between soil moisture and N-efficiency parameters. In addition, the two side dressings of N fertilizer in SPR (140 kg N ha^{-1} each) compared with the seven applications at 45 cm depth (40 kg N ha^{-1} each) in SDI, increased soil NH_4^+ concentration in the 0-10 cm layer, thus possibly promoting N_2O emissions via nitrification and, as a consequence, reducing N-use efficiency (Mosier *et al.*, 1998). Interestingly, HI was lower under SPR-M than under SDI-M treatments in 2019. This was probably due to the common hose reel irrigation management, in which water is supplied in high amounts and with low frequency, thus leading to temporary water stress in plants. If this short, but still significant, water stress matches with high temperature, especially during pollen-shedding and silking stages, the sterile part of the spike will be increased (Hall *et al.*, 1982). Lower irrigation rates coupled with shorter time intervals in SPR, which are uncommon in the region of this study, could have avoided this negative effects.

These results are in contrast with previous findings reporting no benefits of SDI in terms of maize yield compared to sprinkler irrigation (Valentín *et al.*, 2020). However, in this study the authors found also an increase in water use efficiency performances of drip irrigated maize compared with sprinkler, underlining the importance of micro-irrigation systems to increase water productivity of high-demanding water crops. In addition, our findings are in agreement with those found by Hanson and May (2004), observing higher processing tomato yield under SDI than under sprinkler system with similar amount of applied water, and by Zhou *et al.* (2017) reporting higher maize grain yield and nitrogen use efficiency under drip irrigation systems.

We also found that – despite no difference in terms of grain yield – a narrow dripline distance had higher NUE and NUtE than a wider distance in 2019. The lower soil mineral N content for both the 0-10 cm and 10-30 cm soil layers under SDI70-M indicate that narrowing dripline distance from 140 cm to 70 cm can enhance homogeneous spatial distribution of N into the soil. With a wider dripline distance, water and N outflow from a lower number of emitter per unit of soil volume. This may result into temporary "hot-spots" of high soil moisture (above soil water holding capacity), promoting N losses via leaching (and emissions of N as discussed below), and thereby causing a lower N-use efficiency. Indeed, volumetric water content was higher than estimated field capacity (39%) under SDI140-M on 21/06/2019 (42%) and on 25/06/2019 (40%), when fertigations/irrigations occurred.

The benefits for yield and N-efficiency parameters of micro-irrigation systems were not observed in 2020. This was probably because of the higher cumulative rainfall during the growing season of maize in 2020 (347 mm) than in 2019 (194 mm). The calculated ETc for the maize growing season period under SDI treatments was 503 mm in 2020 and 429 in 2019. This means that maize was less dependent on water application via irrigation in 2020, in which only 32% of the crop water requirements was supplied by irrigation (163 mm). On the other hand, the rate supplied with irrigation in 2019 was much higher (243 mm), representing 57% of the total crop water requirement. Therefore, the higher rainfall amount during such a sensitive period in 2020 provided most of the necessary water to support plant growth, and accordingly differences in water application methods became less important for plant yield. Hence, our study highlights that wet years may hinder the benefits for yield potential and N-fertilization efficiency of SDI. Nevertheless, SDI70-M increased iWUE compared with SDI140-M and SPR-M in 2020, confirming its general higher efficiency performances.

Contrary to maize, soybean grain yield was never affected by irrigation technique. This was probably because soybean water requirements are 9-12% lower than those of maize (Brouwer and Heibloem, 2010), which limits the importance of high-efficient irrigation methods. However, the soil NO_3^- content was generally lower in the surface soil under SPR-S than under both SDI treatments. This was probably because irrigation water was supplied with sprinkler at higher rates (per event) than that supplied with SDI, which may have increased water drainage throughout the 0-30 cm soil depth (towards deeper layers), thus increasing N losses through leaching.

4.2 Nitrous oxide emissions as affected by irrigation and N-fertilization method

Micro-irrigation combined with a narrow dripline distance mitigated N_2O emissions compared with sprinkler irrigation, in agreement with previous studies using surface and subsurface drip irrigation methods (Kallenbach *et al.*, 2010; Maris *et al.*, 2015; Wei *et al.*, 2018) and with the recent meta-analyses conducted by Kuang *et al.* (2021) and Yangjin *et al.* (2021). Lower N_2O emission from soil under drip irrigated systems are usually due to partial soil wetting, lower soil moisture, and better temporal/spatial distribution of fertilizers. However, N_2O emissions were not decreased compared to sprinkler irrigation when a wide dripline distance was used. The N_2O reductions with a narrow distance are explained by the lower WFPS and soil mineral N concentration in 0-10 and 10-30 cm soil layers under SDI70-M in 2019. This probably prevented the establishment of anoxic conditions on the one hand, and deprived microorganisms of available N pools on the other hand, thus decreasing N_2O emissions derived from both nitrification and denitrification (Davidson, 1991; Senbayram *et al.*, 2019). As previously discussed, reducing dripline distance to 70 cm promoted a better N exploitation and relocation in grain by providing water and N-fertilizer close to the root zone of each crop row, thus increasing uniformity of input distribution and absorption in time and space (Kallenbach *et al.*, 2010; Maris *et al.*, 2015; Wei *et al.*, 2018). The non-uniform distribution of water and N-fertilizers in soil under SDI140-M may have resulted in the formation of flooded and N enriched “hot spots” near emitters, potentially boosting denitrification (Groffman *et al.*, 2009). For SPR, the double application of urea increased soil mineral N in all soil layers at a higher rate than the numerous applications of ammonium sulphate under SDI70-M. This led to mismatching N availability and plant uptake under SPR (Black *et al.*, 1985; Grant *et al.*, 2012; Xia *et al.*, 2017), thus increasing available N pools for nitrifiers (and subsequently to denitrifiers) right after urea applications (Senbayram *et al.*, 2009).

As hypothesized, cover crop decomposition was affected by the irrigation systems, in turn regulating the emission of N_2O. Both litter and litter N decay rates were higher under SPR compared with SDI70 and SDI140, probably because the application of water on the top of the cover crop residues under SPR promoted microbial activity and litter decomposition (Freckman, 1986; Yahdjian *et al.*, 2006). Conversely, the application of water below the soil surface under SDI treatments avoided soaking the litter, thus reducing microbial activity and fresh organic matter breakdown. Therefore, the higher amount of available C into the soil under SPR than under SDI may have been partly due to higher crop residue decomposition, which seems to be another important factor behind the differences in N_2O between treatments (Weier *et al.*, 1993; Bateman and Baggs, 2005). Interestingly, the contribution of litter and litter-N decay rates to N_2O emissions differed for maize and soybean. Nitrous oxide emissions during maize were associated to (total) litter decay, whereas N_2O emissions during soybean were associated to litter N decay. This suggests that in highly N-fertilized crops (such as maize in our study), the main effect of residue decomposition on N_2O emissions is by providing a C source to denitrifiers (Weier *et al.*, 1993), while the available N released from residue decomposition is less important because there is sufficient N in the soil for soil microorganisms from fertilization. Conversely, for unfertilized crops (such as soybean in our study), N released from litter decomposition may play a major role for N_2O emissions by providing N to nitrifiers and denitrifiers (Senbayram *et al.*, 2019).

Nitrous oxide emissions were higher in 2020 than in 2019 under maize treatments. This was a result of the higher amount of rainfall in 2020 (+79% compared to 2019). The higher rainfall the higher WFPS peaks (81% for SDI70-M and 85% for SDI140-M in 2020, compared to 65% and 72% for the same treatments in 2019), thus stimulating denitrifying microorganisms' activity and therefore N_2O emissions. Further evidence for this mechanism is the strong relationship between cumulative rainfall and N_2O emissions during the growing season of maize.

4.3 Implications for sustainable and efficient management of water and N-supply

Sprinkler irrigation combined with one/two applications of N-fertilizers at a high rate is a widespread agricultural practice due to operational feasibility and reduced labor cost (Black *et al.*, 1985; Grant *et al.*, 2012; Xia *et al.*, 2017). However, our results indicate that this common practice may lead to increased N losses, thus reducing N-fertilization efficiency compared with subsurface drip irrigation. Nevertheless, the agronomic and environmental performance of subsurface drip irrigation varied strongly depending on the crop, dripline distance and growing-season rainfall. Here we show that the benefits of subsurface drip irrigation are higher in crops with high water and N demand, such as maize, than in less demanding crops such as soybean. In addition, within subsurface drip irrigation systems, the choice of dripline distance has a determinant impact on N-fertilization efficiency and partially on yield and iWUE: a narrow dripline distance increases yield, iWUE, and NUE in maize, and this is particularly important during dry years; conversely, during wet years when the contribution of irrigation method is less crucial, dripline distance is less important.

Our results may help defining the amount of rainfall at which using micro-irrigation systems may increase N-use efficiency and yield in fine-textured soils. When around 30% of crop water requirements estimated as ETc are supplied with irrigations, SDI (regardless of dripline distance) may not provide benefits, while when the ratio is around 60%, SDI may increase yield and N-efficiency compared with sprinkler irrigation. This implies that the use of subsurface drip irrigation should be particularly promoted in semi-arid regions, where these systems are already in use. However, since in many temperate areas across the world the climate is changing rapidly towards drier summer seasons (Field *et al.*, 2012), using micro-irrigation systems that minimize water losses through evaporation (i.e. SDI) and increase water use efficiency will become more important in a greater proportion of arable land across the world. Using less water for crop irrigation is crucial to preserve freshwater availability, but also for reducing the C footprint due to the energy required for water extraction, treatment, and distribution (Shrestha *et al.*, 2012).

Our results support the promising outcomes of previous studies with SDI (Patel and Rajput, 2009; Maris *et al.*, 2015; Bronson *et al.*, 2018; Martínez-Gimeno *et al.*, 2018; Gao *et al.*, 2019; Valentín *et al.*, 2020) and show that, when installing a subsurface drip irrigation system for field crops, dripline distance should be designed matching plant spacing, which was 70 cm in the present study, to increase yield performance and reduce negative environmental impacts. However, reducing dripline distance means increasing the number of driplines per hectare, thus increasing investment costs. In addition to lateral spacing, also dripline installation depth may play a major role for steering environmental, productive, and economical performances of field crops. An adequate burial depth depends on several causes, including crop type, soil texture, water source, climate, and cultural practices (Lamm *et al.*, 2006). Thus, long-term studies conducted over several years – performed also in other pedoclimatic conditions and with different crop types – as well as focusing on the interaction between dripline spacing, depth, and installation costs are needed for extending these results at larger scale and for a complete evaluation of SDI system efficiency. These studies should also include lower N and water application rates to explore the potential of sub-optimal amounts to further increase the environmental benefits of SDI. Moreover, future experiments should also use several chambers in a gradient from the dripline to document the spatial variability of N_2O emissions with drip irrigation systems, which may be very large (Abalos *et al.*, 2014).

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5. Supplementary Material

Supplementary material is available as pdf file at the Google Drive address: https://drive.google.com/file/d/1vmOT8v-ZwEldmNIodm_Okn2UYEA3_rO6/view?usp=share_link

CHAPTER 5.

General discussion and concluding remarks

5.1 Effect of resilient practices on crop yield performances

Adopting resilient and climate-smart practices (i.e., no-till, cover cropping, and subsurface drip irrigation) should reduce environmental impact of agriculture while contemporarily sustaining crop yields (Lal, 2004; Kuang *et al.*, 2021). Maintaining current crop productivity at high level is, in fact, mandatory to face a rising food demand due to predicted growth in population in the near future (UN, 2022). Several studies claim that this can be accomplished by focusing on fresh OM return to soil, SOM content preservation/enhancement, and nutrient use efficiency optimization rather than relying on growing application of external inputs (Hawkesford, 2012; Lal, 2015; Fiorini *et al.*, 2020; Boselli *et al.*, 2020). In addition, saving water in agriculture by adopting efficient irrigation schemes (e.g., SDI) has been indicated as a major need (Wang *et al.*, 2019). However, contrasting information is available on crop yield performances of such resilient practices. Some authors reported lower yield performances of NT (Pittelkow *et al.*, 2015) and SDI (Valentín *et al.*, 2020) as compared with conventional practices, including intensive tillage operations and sprinkler irrigation. Moreover, crop yield under NT may be differently affected by diverse cover crop species (i.e., grasses *vs*. legumes), residues of which have different physico-chemical properties.

In Chapters 2 and 3 we investigated either the short- and the long-term effect of sole conservation practices (no-till and cover crops) on crop yield performances, whereas in Chapter 4 we assessed the combined impact of such conservation practices and an efficient irrigation/fertilization method (subsurface drip irrigation with fertigation), as compared with a more traditional irrigation/fertilization scheme (sprinkler irrigation with granular fertilizer application on the soil surface).

In particular, in Chapter 2 we found that NT plus CCs generally reduced maize and soybean grain yield compared with NT without CCs in the short-term. The main reason behind this outcome was mainly ascribed to negative effects of CC residue during the initial phenological phases of main crops. In fact, it is well known that adopting NT may lead to lower soil temperatures and, therefore, to delay plant emergence and rooting in early spring under temperate climate (Wang *et al.*, 2012). Then, combining CCs (i.e. RCC and PM) with relatively high C:N ratio (20-25) and NT may have further boosted this effect, thus ultimately leading to a reduced yield. Conversely, CCs with a low C:N ratio underwent to a fast decomposition, thus limiting the unfavourable effect of reduced soil temperature and crop yield. However, we found also that CCs with an erected habitus after termination may limit yield penalties, but only in case of early termination and with a relatively low residue amount. These results were partially confirmed in the long-term experiment reported in Chapter 3. In this case, no specific pattern of variation in crop yield was found between NT treatments and CT. Nevertheless, NT + rye lowered crop yield of maize and winter wheat during the first years of trial. Beyond lowering soil temperature, retaining cover crop residues with high C:N ratio on the soil surface, may have also caused an increase in soil N-immobilization (Malhi *et al.*, 2001; Burgess *et al.*, 2002; Jin *et al.*, 2008), thus limiting N availability for the following – high N-demanding – crop. Nevertheless, difference in crop productivity between NT and CT treatments was negligible after 5 years of experiment, suggesting that building fertility and restoring soil functions over time through NT and CCs synergic effect may be considered pivotal for maintaining crop yield in the long-term and offset potential negative effects of tillage ceasing.

Once assessed the potential of NT and cover crops to sustain crop yield in the long-term, the next step was to evaluate the effect of different irrigation systems on crop productivity under NT. In Chapter 4, we found that implementing high-efficient irrigation strategies (such as SDI in our experiment) increases crop yield during dry growing season of high water-demanding crops (e.g. maize). This result may be explained by the reduced ETc under SDI than under SPR and by the more efficient water and N-fertilizer distribution, which are supplied together and directly close to the root zone in SDI. However, the benefits of SDI in terms of crop productivity were not observed for lower water-demanding crops (e.g. soybean) in any climate condition and for high water-demanding crops exclusively in wet climate conditions.

5.2 Effect of resilient practices on C sequestration and pools

Regular return of fresh organic matter (from crop residues, cover crops, and/or manure) combined with reduced tillage operations (e.g. no-till) has been indicated as a measure to promote SOC accumulation and soil fertility restoration (Lal, 2015; Boselli *et al.*, 2020; Fiorini *et al.*, 2020). In particular, different CCs may play different agro-ecological functions. Gramineous CCs have the highest potential of biomass production, thus targeting nutrient re-cycling and soil organic matter accumulation (Adetunji *et al.*, 2020), while legume CCs can maximize N input because of biological N-fixation, thus offering the opportunity to increase STN and reduce dependence on chemical N-fertilizers (Fiorini *et al.*, 2022). In addition, brassicaceous CCs are widely recommended as highly-effective catch crops and often indicated as the best choice to remediate soil compaction (Blanco-Canqui and Ruis, 2020). The distinct agro-ecological functions provided by different CC species are partially due to differences in their biomass physico-chemical properties (e.g. lignin content and C:N ratio) (Fageria *et al.*, 2007).

In Chapter 2 we assessed the short-term effect of different CCs species and mixtures on SOC and C pools. Conversely, in Chapter 3 we investigated the long-term effect of no-till coupled with two CCs having opposite C:N ratios (i.e. rye and hairy vetch) on C stabilization in soil aggregates and C sequestration potential and efficiency as compared with conventional tillage without CCs. In detail, we found that the positive effect of CCs on SOC content, due to larger input of fresh residues, is limited to the topmost 5 cm of soil in the short-term under NT, corroborating previous findings (Kern and Johnson, 1993; Koch and Stockfisch, 2006; He *et al.*, 2011). However, our results obtained from the long-term experiment suggest that coupling NT and CCs may increase SOC content even at 5-15 cm depth compared with CT without CCs. The lack of positive effects in the deepest layer under NT+ CCs is mainly due to the limited downward movement of organic material, because crop residues are retained on the soil surface rather than ploughed as for conventional tillage (Boselli *et al.*, 2020). Among CC species, PM was the best option to increase SOC concentration in the short-term, although R and RCC had higher biomass production. This was because of lower C:N ratio of PM residues which have promoted the degradation of the fresh organic material and the inclusion of the deriving C into the SOC through humification (Nicolardot *et al.*, 2001). However, despite having the lowest C:N ratio and high humification rate, VC did not increase total SOC compared with gramineous CCs in the short-term because of the much lower biomass input, underlining the importance of biomass input rate alongside its chemical properties. Regarding C pools, either total extractable C, humic and fulvic acid C, and not extractable organic C were positive related to SOC increase under CC treatments, thus emphasising the scarce contribution of labile C for soil C accumulation in the short-term. Conversely, SOC was increased in both 0-5 cm and 5-15 cm soil layers under NT+CCs treatments compared with CT in the long-term field experiment. In this case, labile form of C greatly contributed to such C accumulation. In detail, the greatest accumulation of SOC and STN under NT+CCs treatments is explained firstly by the larger input of fresh organic residues (because of cover cropping), and secondly by the higher protection from mineralization of SOM within soil aggregates. In fact, C associated with macroaggregates was generally higher under NT-R and NT-V than under CT. This means that labile SOM (e.g. cPOM) is short-term physically protected (until macroaggregate disruption) by creating physical barriers between microorganisms and enzymes and their substrates (Beare *et al.*, 1994; Six *et al.*, 2002), while stable SOM is long-term physically and chemically protected in microaggregates within macroaggregates of which formation is promoted by macroaggregate stability and organic substances availability (Six *et al.*, 2000a; Bandyopadhyay *et al.*, 2010). Results of cPOM and mM-associated C were higher under NT treatments than under CT, thus corroborating higher protection for labile (cPOM-associated) and more stable (mM-associated) SOM. Nonetheless, no difference was found between two cover crops (i.e., rye and hairy vetch) on C sequestration in the long-term. This may be explained by the same amount of biomass and C input provided by both cover crops (Poeplau and Don, 2015). Thus, cover crop biomass production rate (rather than biomass quality) and retention onto the soil as residue could be considered as the main driver of soil C sequestration in the long-term, if N availability is enough to sustain humification processes. Overall, no-till and cover crops showed promising results for climate change mitigation and soil fertility restoration through SOC sequestration, especially in the long-term. In fact, in our study soil accumulated on average 0.5 Mg C ha^{-1} y^{-1} under NT in the 0-30 cm soil layer.

5.3 Effect of resilient practices on N accumulation and on N_2O emissions

Nitrogen (N) is the most important macronutrient for plant growth and metabolism (Mengel and Kirkby, 2001). Since the development of the Haber-Bosch process, N fertilizers are extensively used to address N deficiency in crops, leading to significant N losses especially when N inputs exceed plant needs or soil system capacity (Gruber and Galloway, 2008). NO_3^- leaching and N_2O emissions from agricultural soils are of great concern due to the negative effects on water quality and global warming (Di and Cameron, 2002; Reay *et al.*, 2012). The processes that lead to the formation of such compounds are closely tied with certain farming practices such as soil management, fertilizations, and irrigations (Bowles *et al.*, 2018). Therefore, adopting farming strategies that minimize N losses (e.g. due to mineralization processes), while maintaining crop productivity is mandatory to increase sustainability of agroecosystems. In particular, cover crops may play various role in regulating N cycling into the soil: grasses and brassicas can absorb excess N from the soil (e.g. nitrate), while legumes have the ability to biologically fix N (Kaspar and Singer, 2015). Thus, CCs may be used to reduce environmental impact of agriculture and to provide an important source of organic fresh material and nutrients to soil (Kaye and Quemada, 2017). Moreover, micro-irrigation systems (i.e. subsurface drip irrigation [SDI]) combined with split N-fertilization through fertigation have been suggested as a measure to reduce N_2O emissions from soils and increase N-use efficiency as compared with traditional irrigation techniques (Li *et al.*, 2018; Sandhu *et al.*, 2019; Kuang *et al.*, 2021).

In Chapter 3 we investigated the effect of NT coupled with either rye or hairy vetch on soil N accumulation in comparison with CT, while in Chapter 4 we studied the potential of SDI system to increase N use efficiency of crops and to reduce N_2O emissions from soil. We found that NT and CCs promoted N accumulation into the soil (+88 kg ha^{-1} y^{-1} and +145 kg ha^{-1} y^{-1} for rye and hairy vetch, respectively) due to the larger return of crop residues and to the enhanced aggregate formation and stabilization, thus limiting losses through mineralization (Six *et al.*, 2000). However, we did not find significant differences between rye and hairy vetch in terms of N sequestration rate, although N input from hairy vetch was higher. This may be explained by N susceptibility to losses via NO_3^- leaching and/or N_2O emissions (Cameron *et al.*, 2013) and by the fact that our study was not conducted in N limited environments (average N-fertilization rate was >200 kg N ha^{-1} y^{-1}). In fact, when N-fertilization rate is relatively high, additional N inputs from legume CCs mismatching plant absorption capacity may results in significantly higher N losses as nitrous oxide (N_2O) emissions (Fiorini *et al.*, 2020). Thus, focusing on innovative farming practices that enhance N use efficiency of crops rather than increase N input to the soil may be a viable solution for limit N losses, while sustaining crop yield, in intensive agro-ecosystems (Hirel *et al.*, 2011). In fact, in Chapter 4 we found that using micro-irrigation systems, such as SDI with a narrow dripline distance to match plant spacing, can enhance N use efficiency and mitigate N_2O emissions from soil as compared with sprinkler irrigation. This results are in agreement with previous studies using surface and subsurface drip irrigation methods (Kallenbach *et al.*, 2010; Maris *et al.*, 2015; Wei *et al.*, 2018). The main reason behind higher NUE and lower N_2O emission from soil under drip irrigated systems was due to partial soil wetting, lower soil moisture, and better temporal/spatial distribution of fertilizers. However, N_2O emissions were not decreased compared to sprinkler irrigation when a wide dripline distance was used. The non-uniform distribution of water and N-fertilizers in soil under SDI with a wide dripline distance may have resulted in the formation of flooded and N enriched “hot spots” near emitters, potentially boosting denitrification (Groffman *et al.*, 2009). Based on our findings, the benefits of SDI were substantial under dry climate conditions than under wet climate conditions. Thus, the use of subsurface drip irrigation should be particularly promoted in semi-arid regions, where these systems are already in use. However, since in many temperate areas across the world the climate is changing rapidly towards drier summer seasons (Field *et al.*, 2012), using micro-irrigation systems that minimize water losses through evaporation (i.e. SDI) and increase water use efficiency will become more important in a greater proportion of arable land across the world.

5.4 Conclusions

The main outcomes of this PhD thesis are:

i. Conservation practices (no-till and cover crops) may ensure comparable crop yield to conventional systems especially in the long-term (Chapters 2 and 3), while high-efficient micro-irrigation systems (subsurface drip irrigation) are particularly effective to boost crop yield under dry climate conditions (Chapter 4);
ii. Particular attention should be paid when pairing no-till with high C:N ratio cover crops because of the negative effect on yield due to reduced soil temperatures and increased N immobilization (Chapters 2 and 3);
iii. Large return of fresh organic material through cover cropping and avoiding soil disturbance through no-till may increase C sequestration into the soil by boosting humification processes, stimulating macroaggregate production and physically protecting SOM within aggregates (Chapters 2 and 3);
iv. Labile forms of C scarcely contributed to SOC accumulation in the short-term under no-till, whereas it greatly contributed in the long-term through cPOM protection within macroaggregates (Chapters 2 and 3);
v. The effect of conservation practices on C sequestration was affected by residue biomass quality (C:N ratio) in the short-term, while biomass production rate over residue quality was the main driver for SOC accumulation in the long-term (Chapters 2 and 3);
vi. N accumulation into the soil was not affected by diverse CC species (legumes *vs.* grasses) probably due to N susceptibility to losses via NO_3^- leaching and/or N_2O emission (Chapters 3 and 4);
vii. SDI is a promising irrigation system of which benefits (N use efficiency increase and N_2O emissions reduction) are particularly significant when dripline distance matches plant spacing and when used under dry climate conditions (Chapter 4).

5.5 Future research

In conjunction with findings presented in this thesis, our work suggest a series of topic that would benefit from further research:

i. Assessing the prolonged effect of NT and cover crops on climate change mitigation in diverse pedoclimatic conditions by conducting more long-term studies;
ii. Evaluating the effect of periodic tillage events (e.g., subsoiling), which are occasionally used as practice to reduce possible NT unfavorable effect;
iii. Focusing on different levels of N-fertilization to assess optimal balance between CC-derived N inputs and N-fertilizer rates, thus aiming to avoid surplus and losses;
iv. Studying the interactions between dripline spacing, depth, and installation costs for extending the results at larger scale and for a complete evaluation of SDI system efficiency

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