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PHD COURSE in: Crop Science

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**SUSTAINABLE LAND MANAGEMENT PRACTICES IN THE LOW-LYING VENETIAN  
PLAIN: RELATIONSHIP TO SOIL ECOSYSTEM SERVICES**

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## **Declaration**

I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree or diploma of the university or other institute of higher learning, except where due acknowledgment has been made in the text.

Legnaro 30/09/2019

Carlo Camarotto

A handwritten signature in black ink, reading "Carlo Camarotto". The signature is written in a cursive style with a prominent initial 'C'.

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## Summary

Sustainable land management (SLM) practices, as conservation agriculture (CA) and conventional tillage with cover crops (CC), aimed at balancing competitive agricultural production and environmental protection, have been encouraged throughout the EU through policy and subsidisation. Adoption of SLM practices that regulate biogeochemical cycles, however, requires further study, especially given the effects of local pedo-climatic variability and because middle and long-term effects are not fully understood and may differ from short-term outcomes.

For these reasons, in this work, field experiments were conducted in three farms in the low-lying venetian plain, characterized by loamy soils, where CA and CC were compared to conventional intensive tillage system (CV) on trials established since 2010.

The first objective of the thesis was to evaluate, by integrating experimental field results with model predictions, the potential ecosystem services provided by CA and CC practices on SOC dynamic, air quality and climate regulation, nutrition biomass and regulating of water conditions. In this experiment, CA and CC results contrasted according to the soil functions, the ecosystem service category and evaluation time span. The former was more effective in providing regulating services in the short term, and less consistent in the long term, at least for GHG mitigation. GHG control is only one of the numerous ecosystem services provided by conservation practices (e.g. reduction of erosion and P particulate loss). Many of these depend on the C content which are strongly affected by the C stratification processes. Cover crop adoption, on the contrary, showed promise in the long term, whereas short-term outcomes (two-year experiment) were negatively affected by poor cover crop growth.

The second objective aimed to assess the SOC stock variation due to the adoption of CA and CC in comparison to CV within a large sample (i.e., 240) of 0-50-cm soil profiles, comparing two expansive soil sampling operations conducted in 2011 and 2017. The study showed that CA enhances SOC stratification rather than SOC accumulation, with high topsoil SOC that may have partly counteracted soil surface compaction. However, a comparison with previous SOC stock quantifications between CA and CV after three years of the experiment suggests that some SOC stock increase occurred, even at 50 cm, despite being not significant. The burial of fresh biomass-C with cover crops in arable systems (CC) enhanced SOC stock depletion most likely due to priming effects, suggesting that C input management is pivotal for its accumulation in agroecosystems with low soil fertility and low SOC protection capacity.

# **Chapter I - General background**

## Cover Cropping and Conservation Agriculture as Sustainable Land Management (SLM) Practices

The relationship between agriculture and the environment has always been at the centre of the international debate aimed at sustainable development, since there is a close link in the agronomic field between the natural resources to be protected and their exploitation. Globally, it is estimated that less than a quarter of the world's surface has not yet been altered by human action (Ellis & Ramankutty, 2008) and that agriculture alone occupies about 11% of the Earth's surface (Dubois, 2011), using about 70% of the planet's water resources and contributing about 13.5% of total greenhouse gas emissions (IPCC, 2007). It is also estimated that 52% of the land used for agricultural purposes is moderately or severely affected by soil degradation. In 2008, agricultural soil degradation, mainly due to intensified management practices (Gibbs & Salmon, 2015), directly affected 1.5 billion people (Bai *et al.*, 2008). The link between environmental protection, agriculture and soil is evident in many Sustainable Development Goals (SDG), as defined by the United Nations Agency in Agenda 2030 (UN, 2015), in which agricultural soil plays an important role in addition to the strictly agronomic-productive aspects. It involves also the health and well-being of mankind through the restoration and maintenance of quality water resources, the contribution to the fight against climate change and much more (Figure 1).

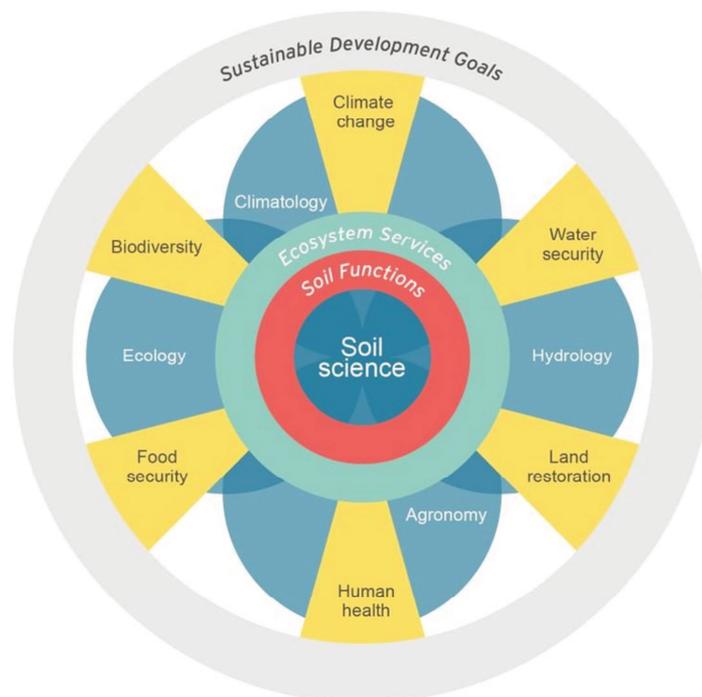


Figure 1 Link among soil, ecosystem services and Sustainable Development Goals (Keesstra *et al.*, 2016).

The new global vision for sustainable agricultural land management is now noticeable at all levels of legislation: European, national and regional. In Veneto (NE Italy), among the priorities set in the last two rural development plans (Veneto Region, 2013; Veneto Region, 2015) there are those of preserving, restoring and enhancing the ecosystems related to agriculture, undertaking a process of enhancement and encouragement of agricultural practices defined as sustainable through payments for the adoption of defined agri-environmental measures. Among these agronomic practices, those that provide a continuous coverage of the soil with conventional tillage (CC), and the conservation agriculture (CA), in which the continuous coverage is associated with a minimization of soil tillage and crop rotations (Lal, 2004; Verhulst *et al.*, 2010; Branca *et al.*, 2011), have been considered efficient.

Permanent soil cover is usually achieved through the retention of crop residues on the surface and through the use of cover crops (Vanepf & Benites, 2001). Cover crops are plants grown in agroecosystems for the ecosystem services they provide rather than a harvestable product (i.e., food, fuel, fiber - with the exception of forage and feed); thus, they may also be called “service crops” since their primary purpose is to provide diverse services including soil cover and nutrient scavenging (Ogilvie *et al.*, 2019). They are considered important to enrich the soil with organic matter, improve soil fertility (in particular by using legumes as cover crops), increase soil bearing capacity, reduce erosion and leaching, promote biodiversity and, combined with crop rotation, interrupt the pest cycle (Witmer *et al.*, 2003; Thierfelder & Wall, 2009; Farooq & Siddique, 2015). Furthermore, cover crops can alter the water balance in agroecosystems by facilitating transpirational water loss, reducing evaporative water loss, facilitating water infiltration, modifying soil water storage and holding capacity, and enhancing the subsequent crop’s ability to access soil water (Figure 2) (Ogilvie *et al.*, 2019).

Minimal soil disturbance (in particular no-tillage), instead, is related to numerous improvements due to the absence of soil fragmentation (Soane *et al.*, 2012). Greater aggregate stability is reported (Six *et al.*, 2002), due to both an increase in soil organic carbon (SOC) stock (West & Post, 2002) and a higher fraction of stable SOC (McCallister & Chien, 2000; Bayer *et al.*, 2003), greater carbon sequestration (Lal & Kimble, 1997), with positive effects on air quality with respect to greenhouse gases and, therefore, useful to combat climate change. Finally, the lack of tillage also has a positive impact on the habitat and activity of the soil flora and fauna (Blackwell *et al.*, 1990; Horn, 2004; Causarano *et al.*, 2008).

Despite the above, however, neither CC nor CA are still universally recognized as win-win solutions for the agro-ecosystem improvement, given that many authors have raised doubts about

the real productive and ecological benefits that the two practices can bring (Powlson *et al.*, 2014; VandenBygaart, 2016). Depending on the water cycle (e. g. amount of rain, drainage) and the period of establishment, CC can adversely affect agricultural production by removing water and immobilising nutrients (Thorup-Kristensen *et al.*, 2003). Some research found decreases in SOC stocks with cover crops use (Poepflau & Don, 2015), while under no-tillage we may have a different distribution of the SOC along the profile, rather than to an increase in absolute values (Powlson *et al.*, 2011). Many authors have also found that the absence of tillage has a negative impact on soil bulk density (Dal Ferro *et al.*, 2014; Palm *et al.*, 2014) and on its structure (Munkholm *et al.*, 2013). Surface compaction, considered to be one of the main reasons for yield reduction under no-tillage (Carter, 1991; Ball *et al.*, 1994), inhibits deep root growth (Baker *et al.*, 2007), which is essential to obtain a stable soil organic pool (Rasse *et al.*, 2006). Declines in yields have often been attributed to cool and wet climatic conditions (Ogle *et al.*, 2012), and, in general, the overall benefits of CA have been closely related to soil type and climate (Soane *et al.*, 2012). Soils with low structural stability and low SOC content, such as those in the low-lying venetian plain, may be more susceptible to compaction (Van Ouwerkerk & Perdok, 1994; Munkholm *et al.*, 2003), and therefore less suitable for no-tillage practice. Finally, medium and long-term effects may differ from those found in the short term (Constantin *et al.*, 2010; Piccoli *et al.*, 2017). Therefore, the soil-water-crop dynamics are still not fully understood, due site-specific soil-related reactivity to management changes and inherent variability in the experiments (e.g., changes in climatic conditions).

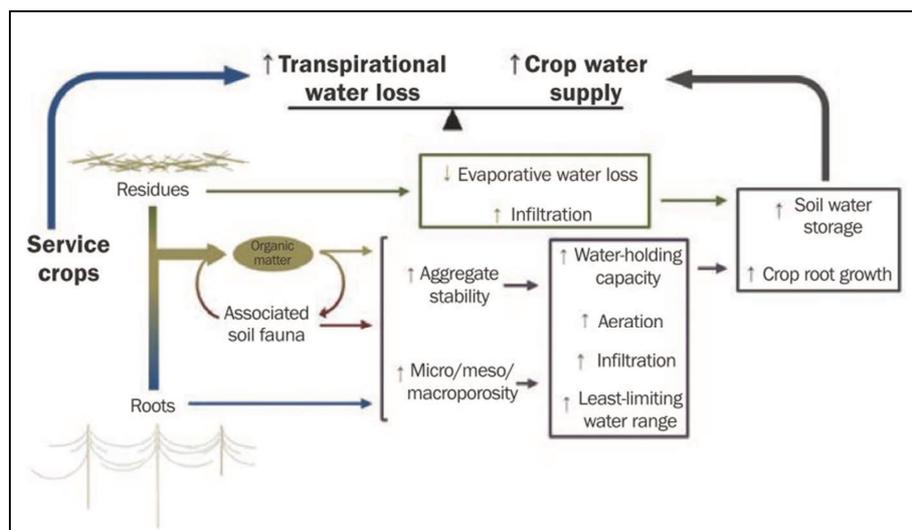


Figure 2 Summary of potential cover crop impacts on agroecosystem elements that alter the crop water supply–transpirational water loss balance (Ogilvie *et al.*, 2019).

## Thesis objectives and outline

Given the above-mentioned lack of coherence of the results, further research is necessary to understand the real potential of CA and CC as sustainable agronomic practices, especially by adapting them to the particular soil and climate conditions of Veneto and carrying out long-term experiments, in order to identify possible transitional effects.

For these reasons, in this work, field experiments were conducted in three farms in the low-lying venetian plain, characterized by loamy soils, where CA and CC were compared to conventional intensive tillage system (CV) on trials established since 2010.

The first objective of the thesis was to evaluate, by integrating experimental field results with model predictions, the potential ecosystem services provided by CA and CC practices on SOC dynamic, atmospheric composition and climate regulation, nutrition biomass and regulating of water conditions. In a single farm (on three fields), 17-month recordings from three soil-water monitoring stations per treatment (9 in total) were combined with climatic data to estimate water and N fluxes in the 0-60 cm layer. Carbon fluxes were quantified considering SOC and biomass contents. The biogeochemical model DeNitrification DeComposition (DNDC) was employed to evaluate long-term (105-yr) carbon dynamics and quantify greenhouse gas (GHG) emissions as affected by SLM practices and climate conditions.

The second objective aimed to assess the SOC stock variation due to the adoption of CA and CC in comparison to CV within a large sample (i.e., 240) of 0-50-cm soil profiles, comparing two expansive soil sampling operations conducted in 2011 and 2017. We hypothesized that in comparison to CV, minimum mechanical soil disturbance, maintenance of permanent soil covering and crop diversification can enhance SOC stocks by offsetting in a six-year period the slow reaction capacity and poor SOC protection mechanisms of the Veneto plain soils.

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# Chapter II - Conservation agriculture and cover crop practices to regulate water, carbon and nitrogen cycles in the low-lying Venetian plain\*

\* **Camarotto, C.**, Dal Ferro, N., Piccoli, I., Polese, R., Furlan, L., Chiarini, F. and Morari, F. (2018) *Conservation agriculture and cover crop practices to regulate water, carbon and nitrogen cycles in the low-lying Venetian plain*. Catena, 147 (2018), pp. 236-249.

## 1. Introduction

There is growing interest in Europe to establish sustainable land management (SLM) practices that provide ecosystem services beyond maximising crop yield (Maier and Shobayashi, 2001; Van Zanten *et al.*, 2014). The Rural Development Programme (RDP) and agri-environment schemes finance SLM practices to favour protection, conservation, and improvement of natural resources (soil, water and air), biodiversity, and rural area landscape and cultural heritage (Uthes and Matzdorf, 2013). Practices that provide continuous soil cover (e.g., cover crops) and minimal soil disturbance (e.g., reduced or no-tillage) of arable lands have been supported in more than 50% of RDPs at the EU27-level (Keenleyside *et al.*, 2011; Zimmermann and Britz, 2016). It is well known that the primary function of cover crops (CC) is to tighten the nitrogen cycle, especially in the short term, by reducing nitrate leaching and by acting as a green manure (Constantin *et al.*, 2010; Gabriel and Quemada, 2011). Nevertheless, depending on the water cycle (e.g., amount of rainfall, drainage) and period of establishment, CC may also negatively affect crop production by subtracting water and immobilising nutrients (Thorup-Kristensen *et al.*, 2003). A secondary role of cover crops is to increase soil organic carbon (SOC) stocks, and in turn, soil fertility of croplands (Poeplau and Don, 2015), although the debate of relative effectiveness of cover crops versus other practices (e.g., minimal soil disturbance, incorporation of organic amendments) continues.

Conservation agriculture (CA) is a system of agronomic practices that minimises mechanical soil disturbance, maintains permanent soil cover by using crop residues and cover crops, and includes crop rotation (Farooq and Siddique, 2015). It has received wide attention as a way to reverse the decline in soil functions experienced in intensive agricultural systems, such as SOC stock depletion, microorganism habitat loss, and nutrient cycling imbalances, which make food and feed production unsustainable in the long term (Verhulst *et al.*, 2010). Alternatively, CA can negatively or positively affect soil structure properties (e.g., bulk density, soil strength) depending on local context (Soane *et al.*, 2012). In particular, while a change in soil hydrology is usually expected, some authors (e.g., Palm *et al.*, 2014) found CA enhanced water infiltration from structure stability and bio-macropore connectivity (i.e., wormhole) improvements, while Lipiec *et al.* (2006) reported compromised water infiltration (-61%) due to high traffic soil compaction. Moreover, higher soil moisture content from crop residue mulching (Liu *et al.*, 2013) also offsets cover crop water consumption (Thorup-Kristensen *et al.*, 2003), which can be critical in rain-fed systems.

Considering the complexity of agro-ecosystems and quantification of their services, it is not surprising that simulation models combined with field studies have been used increasingly to

improve predictions of agro-environmental indicators. Models to predict GHG emission have been developed, as have biogeochemical models that integrate several management and pedo-climatic factors in sub-models (e.g., biomass production, grain and nutrients allocation, soil-water dynamics, C and N flows) in an attempt to quantify the agronomic and environmental outcomes associated with the adoption of different SLM practices (Xu *et al.*, 2013; Cui *et al.*, 2014).

Despite the growing attention of scientists and policymakers with economic incentives to encourage adoption of SLM practices, CC and CA use among European farmers remains weak (Basch *et al.*, 2015; Bergtold *et al.*, 2017). Other than direct compensation to farmers for adopting SLM practices, farmers remain uncertain of their ability to match the dual challenges of maintaining economic viability and improving environmental quality. Two reasons inform this predicament of further adoption. First, too little attention has been paid to the effect of pedo-climatic variability on SLM effectiveness to guarantee balanced ecosystem service trade-offs (Power, 2010; Primdahl *et al.*, 2010). Second, middle and long-term effects are not fully understood and may differ from short-term outcomes (Constantin *et al.*, 2010; Piccoli *et al.*, 2017).

In Veneto region (northeast Italy), both conservation agriculture and cover crops were subsidised and adopted during the 2013 and 2015 RDPs (Regione Veneto, 2015; Regione Veneto, 2013) on an area representing about 1% of the region's arable land (Dal Ferro *et al.*, 2016). However, with the aim to increase their implementation, CC and CA were selected as promising land management practices after a participatory process that engaged stakeholders under the EU FP7 project "RE CARE – Preventing and Remediating degradation of soils in Europe through Land Care" (<http://www.recare-project.eu/>). The general goal of RE CARE in the study area is to reverse the degradation of mineral soils of Veneto that generally have low SOC content.

By integrating experimental field results with model predictions, this study aims to evaluate the potential ecosystem services provided by conservation agriculture (CA) and cover cropping (CC) practices on SOC dynamic, atmospheric composition and climate regulation, nutrition biomass and regulating of water conditions.

## 2. Material and methods

### 2.1. Study area

The experiment was conducted on a farm located in the southwest of the low-lying Venetian plain ( $45^{\circ} 2.908' N$ ,  $11^{\circ} 52.872' E$ , 2 m a.s.l.) (Figure 3), characterised by a water table level ranging from about -250 cm in summer to -70 cm in winter. The soil is silty-loam Endogleyc Cambisols (FAO-UNESCO, 1990) and of medium fertility due to its relatively low SOC concentration ( $1.2 \text{ g } 100 \text{ g}^{-1}$ ) (Table 1). The sub-humid climate receives an annual rainfall of 673 mm that is uniformly distributed throughout the year (129 mm in winter and 187 mm in autumn). Temperatures rise between January ( $-0.2 \text{ }^{\circ}\text{C}$  minimum average) and July ( $30.6 \text{ }^{\circ}\text{C}$  maximum average), and the 848 mm reference evapotranspiration ( $ET_0$ ) exceeds rainfalls between May and October with a maximum in July ( $4.8 \text{ mm d}^{-1}$ ).

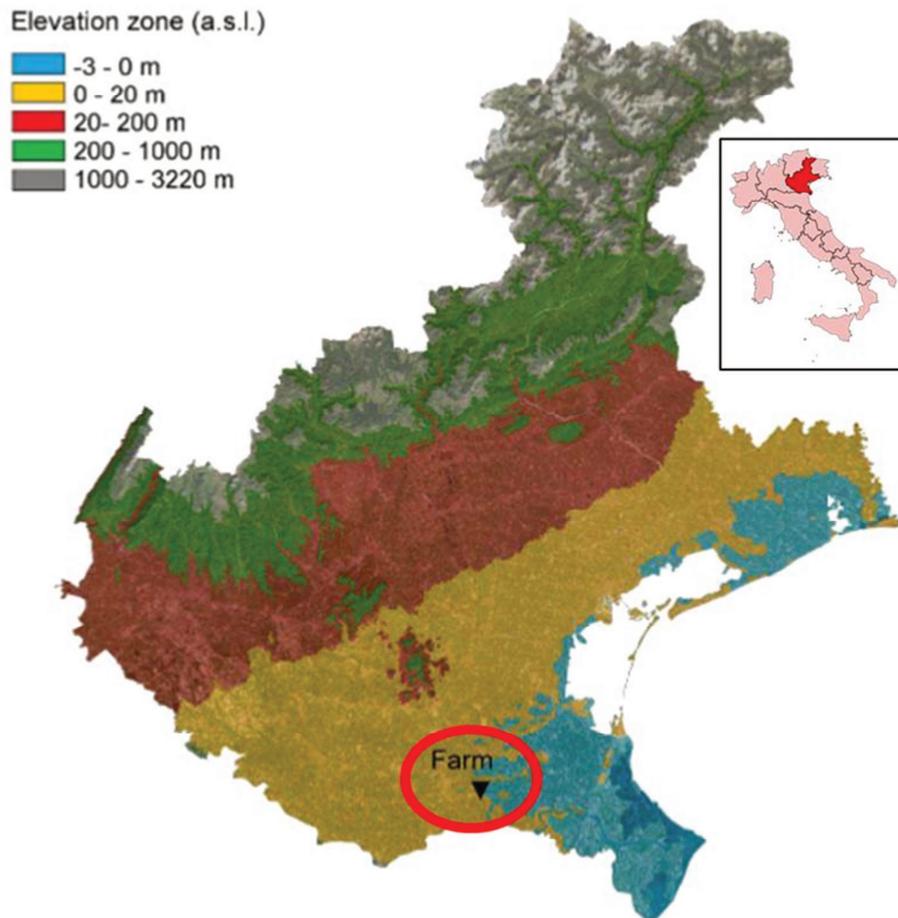


Figure 3 Experimental site in Veneto Region low plain, north-eastern Italy.

Treatment	Layer cm	Sand (50-200 $\mu\text{m}$ ) (g 100 g <sup>-1</sup> )	Silt (2-50 $\mu\text{m}$ ) (g 100 g <sup>-1</sup> )	Clay (< 2 $\mu\text{m}$ ) (g 100 g <sup>-1</sup> )	SOC (g 100 g <sup>-1</sup> )	Total nitrogen (g 100 g <sup>-1</sup> )
CA	0-5	16.4 ( $\pm 2.9$ )	58.6 ( $\pm 1.0$ )	25.0 ( $\pm 2.3$ )	2.13 ( $\pm 0.20$ )	0.31 ( $\pm 0.02$ )
	5-30	15.1 ( $\pm 2.8$ )	59.5 ( $\pm 1.3$ )	25.4 ( $\pm 2.7$ )	1.07 ( $\pm 0.11$ )	0.20 ( $\pm 0.01$ )
	30-50	14.7 ( $\pm 3.1$ )	59.3 ( $\pm 1.8$ )	26.0 ( $\pm 2.7$ )	0.99 ( $\pm 0.08$ )	0.19 ( $\pm 0.01$ )
CC	0-5	21.6 ( $\pm 3.1$ )	53.0 ( $\pm 1.0$ )	25.4 ( $\pm 2.2$ )	1.51 ( $\pm 0.13$ )	0.24 ( $\pm 0.01$ )
	5-30	15.7 ( $\pm 3.8$ )	57.7 ( $\pm 1.9$ )	26.6 ( $\pm 2.1$ )	1.21 ( $\pm 0.10$ )	0.21 ( $\pm 0.01$ )
	30-50	19.5 ( $\pm 3.6$ )	54.1 ( $\pm 1.4$ )	26.4 ( $\pm 2.3$ )	1.01 ( $\pm 0.12$ )	0.20 ( $\pm 0.01$ )
CV	0-5	14.0 ( $\pm 2.0$ )	59.0 ( $\pm 1.0$ )	27.0 ( $\pm 2.6$ )	1.17 ( $\pm 0.17$ )	0.22 ( $\pm 0.01$ )
	5-30	13.6 ( $\pm 2.4$ )	57.8 ( $\pm 0.7$ )	28.6 ( $\pm 2.6$ )	1.25 ( $\pm 0.12$ )	0.23 ( $\pm 0.01$ )
	30-50	13.2 ( $\pm 2.2$ )	59.4 ( $\pm 1.2$ )	27.4 ( $\pm 2.4$ )	1.04 ( $\pm 0.09$ )	0.21 ( $\pm 0.01$ )

Table 1 Average soil properties in the three treatments (standard error in brackets).

## 2.2. Experimental design and treatments

The field experiment established in October 2010 and still underway compares a conventional agricultural (CV) system with cover crop (CC) and conservation agriculture (CA) managements. CC and CA systems were set-up per Agri-environmental Measures 214 – Sub-Measure “i” (also called “Eco-compatible management of agricultural lands”) of the Rural Development Plan for the Veneto Region during the period 2007–2013 (Regione Veneto, 2013) stemming from European Council Regulation (EC) No 1698/2005. Study lay-out consists of three rectangular adjacent plots (average size: 1.62 ha, about 540 m length  $\times$  30 m width), one for each specific treatment.

The same four-year crop rotation of winter wheat (*Triticum aestivum* L.) – oilseed rape (*Brassica napus* L.) – soybean (*Glycine max* (L.) Merr.) – maize (*Zea mays* L.) was initially used for all treatments. In 2015, the rotation was successively simplified to three years when oilseed rape cultivation was abandoned. In CA and CC, continuous soil cover was accomplished via cover crop inter-cropping with sorghum-sudangrass (*Sorghum  $\times$  drummondii* (Nees ex Steud.) Millsp. & Chase) in the spring-summer season and winter wheat in the autumn-winter season. These crops replaced a vetch and barley mixture (*Vicia sativa* L. and *Hordeum vulgare* L.) used during the first four experimental years. Conversely, the soil remained bare between the main CV crops.

In CV and CC systems, crop residues and cover crops acting as green manure (in CC only) were incorporated 35 cm into the soil with a multi-board plough, and their seedbeds were prepared by disk harrow to 15 cm in depth. System CA was managed with no-tillage, cover crop devitalisation, direct sowing, harvesting with crop residues left on the soil surface, and cover crop sowing.

The fertiliser base dressing was applied one to two weeks before sowing in CC and CV, whereas sub-surface band fertilisation was applied to CA during sowing. All systems were side-dressed with

mineral fertilisers one time in maize and two times in wheat. As specified in the protocol (Table S1), no additional fertilisation was provided to the cover crops. In winter wheat, NPK mineral fertilisation was provided at doses of 32 kg N ha<sup>-1</sup>, 96 kg P-P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>, and 96 kg K-K<sub>2</sub>O ha<sup>-1</sup>. In soybean, only phosphorus (50 kg P-P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>) and potassium (50 kg K-K<sub>2</sub>O ha<sup>-1</sup>) were applied as mineral fertilisers. Maize received compound mineral input (32 kg N ha<sup>-1</sup>, 96 kg P-P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>, 96 kg K-K<sub>2</sub>O ha<sup>-1</sup>) followed by urea (69 kg N ha<sup>-1</sup>) at sowing (1-10 April in CV and CC, 10-20 April in CA). Side dressing treatments are performed in maize as urea (115 kg N ha<sup>-1</sup>) and in wheat as ammonium nitrate (50 kg N ha<sup>-1</sup>) and urea (92 kg N ha<sup>-1</sup>).

Pesticide applications based on crop requirements followed an integrated pest management programme and were the same for CV, CC, and CA. Prior to spring seeding, N-(phosphonomethyl) glycine was applied to suppress winter cover crop in CA, while mechanical shredding was utilised to suppress winter cover crop in CC. Sorghum-sudangrass was mechanically suppressed in both SLM managements.

### 2.3. Data collection

As of March 2016, nine soil-water monitoring stations were installed in the experimental fields (CV, CC, and CA with three stations each). Each monitoring station was equipped with multi-sensor probes (HD3510.2, Delta OHM, GHM GROUP, Selvazzano Dentro, IT), suction lysimeters (60 cm depth) (Soilmoisture Equipment Corp., Santa Barbara, CA, USA), and phreatic wells (350 cm depth) to study the effects of different treatments on soil-water dynamics and nitrogen balances.

The multi-sensor probes continuously monitored soil temperatures (T, °C) and volumetric water content (VWC, m<sup>3</sup> m<sup>-3</sup>) at three depths (10, 30, and 55 cm). Prior to field installation, the soil moisture sensors, operating with frequency domain reflectometry (FDR) technique, were calibrated in the laboratory to an accuracy of ±3%. Data were recorded every 15 min and regularly monitored by a radio frequency wireless remote control system using ISM radio bands. The system connected the monitoring probes to a weather station (Delta OHM, GHM GROUP, Selvazzano Dentro, IT) via GSM technology. The weather station was equipped with a thermometer, hygrometer, anemometer, pyranometer, and rain gauge. Soil water quality and water table depths were monitored biweekly during the 17-month trial (April 2016 - August 2017).

Phreatic wells consisted of a 5-cm diameter polyvinyl chloride tube with a slotted polyvinyl chloride screen in the lower 1 m. The annulus around the screen was filled with calibrated gravel and the first 10 cm depth was sealed with bentonite clay. The suction lysimeters consisted also of a

5-cm diameter polyvinyl chloride tube with a cup of ceramic porous material placed at its bottom end. Water collected in both the phreatic wells and lysimeters were analysed in the laboratory for nitrate in solution (CNR-IRSA, 1994).

Crop yield and residue samples, taken from three 2-m<sup>2</sup> areas in each treatment, were collected and dried at 65 °C in a forced draft oven for 72 h for dry weight determination. Aboveground cover crop biomasses (sorghum-sudangrass during spring-summer; winter wheat during autumn-winter) were sampled this time from three 1-m<sup>2</sup> areas before suppression in CA and soil incorporation in CC. Sample dry biomass weights were then determined after oven drying for 72 h at 65 °C.

To evaluate the effects of continuous soil cover by crop residues and cover crops, and of undisturbed soil management on SOC stocks, we chose a six-year interval as appropriate to account for the slow reaction of SOC to land use changes. Two soil-sampling campaigns were undertaken, the first in spring 2011 and the second in spring 2017. Specifically, a hydraulic sampler was used to take undisturbed soil cores (0-50 cm) from six systematically chosen locations in each field. The same locations were identified across the years using global navigation satellite system with Real-Time Kinematic differential correction (*ca.* 2 cm precision). The soil core samples were cut into three distinct layers of 0–5 cm, 5–30 cm, and 30–50 cm, and then stored at 5 °C for later physical and chemical analysis. A total of 108 undisturbed soil samples (3 treatments × 2 dates × 3 layers × 6 sampling points) were weighed; a fraction (two-thirds) of these was oven-dried at 105 °C for 24 h from which the bulk density was calculated. To determine organic carbon and nitrogen content, the other fraction (one-third) was air-dried and sieved through 0.5 mm mesh. Flash combustion using a CNS elemental analyser (Vario Max, Analysensysteme GmbH, Langenselbold, DE) was employed following the removal of inorganic carbon with an acid pre-treatment. To quantify the SOC stock, which could be confounded by the effects of tillage on bulk density, we used the equivalent soil mass method (VandenBygaart and Angers, 2006). Finally, soil texture was determined with laser diffractometry (Malvern Mastersizer 2000, Malvern Instruments, Malvern, UK) of 2-mm sieved samples that had been previously dispersed in a 2% sodium hexametaphosphate solution and shaken for 12 h at 80 rpm.

#### **2.4. Water and nitrogen mass balance**

Soil and weather data were used to estimate both water and nitrogen fluxes according to Morari *et al.* (2012) in the two-cropping season period from April 2016 - August 2017. The water balance

method was applied to calculate recharge (upflux) and/or drainage (percolation),  $P$ , in the 0-60 cm layer as follows:

$$P = R - ET \pm \Delta W_t, \quad [1]$$

where  $R$  is rainfall (mm),  $ET$  is crop evapotranspiration (mm) (Allen *et al.*, 1998), and  $\Delta W_t$  is the soil water storage difference (mm) in the 0-60 cm layer, all calculated on a daily basis. Runoff was neither monitored, nor included in the water balance, however, previous studies conducted in the same area have reported negligible runoff (< 2% of total outflow) (Morari *et al.*, 2012).

Nitrogen mass flux ( $M_N$ , kg ha<sup>-1</sup>) that entered by upflux or leached into the 0-60 cm soil layer was estimated at each lysimeter during time interval  $T$  according to the equation by Green *et al.* (2008):

$$M_N = \int_0^T P(t)C_N(t)dt, \quad [2]$$

where  $P(t)$  (m d<sup>-1</sup>) is the vertical flux of water (drainage or upflux) at the lower boundary (60 cm depth) and  $C_N(t)$  is the nitrogen concentration (kg m<sup>-3</sup>). Equation (2) was integrated according to the trapezoidal rule proposed by Lord and Shepherd (1993) as water was generally collected biweekly:

$$M_N = \sum_i P_i C_{N,i}, \quad [3]$$

where  $P_i$  and  $C_{N,i}$  are vertical water flux and average nitrogen concentration during time interval  $i$ .

Nitrogen mass balance was then calculated per the following equation:

$$N_{bal} = N_{fert} + N_{rain} + N_{up} - N_{uptake} - N_{leach}, \quad [4]$$

where  $N_{bal}$  (kg ha<sup>-1</sup>) represents a combined term that includes N air losses (volatilisation and denitrification) and the change in N content of the soil profile between the end and the beginning of the monitored period.  $N_{fert}$ ,  $N_{rain}$ , and  $N_{up}$  (kg ha<sup>-1</sup>) are N inputs (fertilisers, rainfall, and upflux, respectively);  $N_{uptake}$  (i.e., exiting the field with crop yields) and  $N_{leach}$  (i.e., exiting the 0-60 cm layer, estimated from suction lysimeters) (kg ha<sup>-1</sup>) are N outputs..

## 2.5. DNDC agro-ecosystem model

To estimate gas emissions for a two-year period (2016-2017) and a 105-year period (2018-2122), as well as SOC stock variation over the 105 years, the DNDC model was applied to the three management systems while taking into account climate change scenarios. DNDC (version 9.3) is a

process-based biogeochemical model originally designed to estimate N<sub>2</sub>O emissions from agricultural systems (Li *et al.*, 1992). It has been successively updated to estimate carbon (e.g., SOC dynamics, CO<sub>2</sub> emissions) and nitrogen (e.g., NH<sub>3</sub> emissions, nitrate leaching) transportation and transformation in the plant-soil system. The model, consisting of six interacting sub-models that simulate soil climate, plant growth, decomposition, nitrification, denitrification, and fermentation, has been successfully applied to many agro-ecosystems worldwide (Cui *et al.*, 2014; Li *et al.*, 2014; Chen *et al.*, 2018). The field experiment provided the input parameters required for the model: daily weather data (e.g., temperature and rainfall), soil properties (e.g., soil density, texture, initial SOC), land use (e.g., crop type and rotation system), and management practices (e.g., tillage, fertilisations, irrigation, and crop residue management).

## 2.6. DNDC model validation

Preliminary validation of the DNDC model was necessary to assess its reliability and sensitivity to different agronomic and pedo-climatic conditions. Data from the experimental fields during the six-year (2011-2017) time span (i.e., crop yields, total aboveground biomass, SOC) and 17-month monitoring period (i.e., daily average soil moisture at different depths, N content in percolation water) were used to validate the model. Model prediction uncertainty, stemming from imperfect knowledge of input parameters (Giltrap *et al.*, 2010), was quantified using Monte Carlo simulations derived from soil property variability in the fields. For a set interval (i.e.,  $\pm$  standard deviation)—and with random selection from the data inputs of bulk density, SOC concentration, and clay content—DNDC generated 512 random combinations and predicted SOC concentration values to quantify the likely range and distribution of output data (Li *et al.*, 2004). Furthermore, simulated grain yields, SOC concentrations, soil moisture levels, and nitrate concentrations in leached water were compared with observed data. To test the goodness of fit between model outcomes and field data, the index of model efficiency (EF), Theil's inequality ( $U^2$ ), and the coefficient of determination ( $R^2$ ) were all calculated (Tonitto *et al.*, 2010). Model outcomes produced good approximations of field data when both EF and  $U^2$  are in the range  $0 \leq x \leq 1$ , with EF = 1 and  $U^2 = 0$  representing the best fit.

The DNDC model estimated crop yields well during the six-year field experiment, as shown by measures of very high model efficiency (EF= 0.90) and low inequality ( $U^2= 0.020$ ) between the predicted and observed values (Figure 4). Crop production was slightly overestimated in CC and CA only in the rainy years (2013, 2014, and 2016; average rainfall 915 mm); conversely, a slight underestimation occurred in the most dry years of 2011, 2012, and 2017 (average rainfall 548 mm).

In general, the DNDC model also produced good estimates of SOC concentrations measured in 2017, and demonstrated a high model efficiency ( $EF = 0.67$ ) and low inequality ( $U^2 = 0.023$ ) between predicted and observed values (Figure 5). The only underestimation was of SOC in the topsoil layer under CA, for which  $1.65 \text{ g C } 100 \text{ g}^{-1}$  was predicted and  $2.13 \text{ g C } 100 \text{ g}^{-1}$  was the actual experimental result. The DNDC simulated water contents well at the shallow depth (10 cm), but were less accurate in CV and at greater depths (30 and 50 cm) ( $EF < 0$ ). The low efficiency of the DNDC model to simulate CV was found after deep ploughing operations in November 2016 ( $EF = 0.54$  and  $-1.69$ , before and after ploughing, respectively). The 50 cm layer in all three treatments was difficult to represent in the DNDC model when the water table was shallow. The comparison between observed and predicted nitrate concentrations in leached water did not provide satisfactory results. On average,  $EF$  was negative ( $-0.35$ ), whereas Theil's inequality index was satisfactory ( $U^2$ ) within the range 0-1.

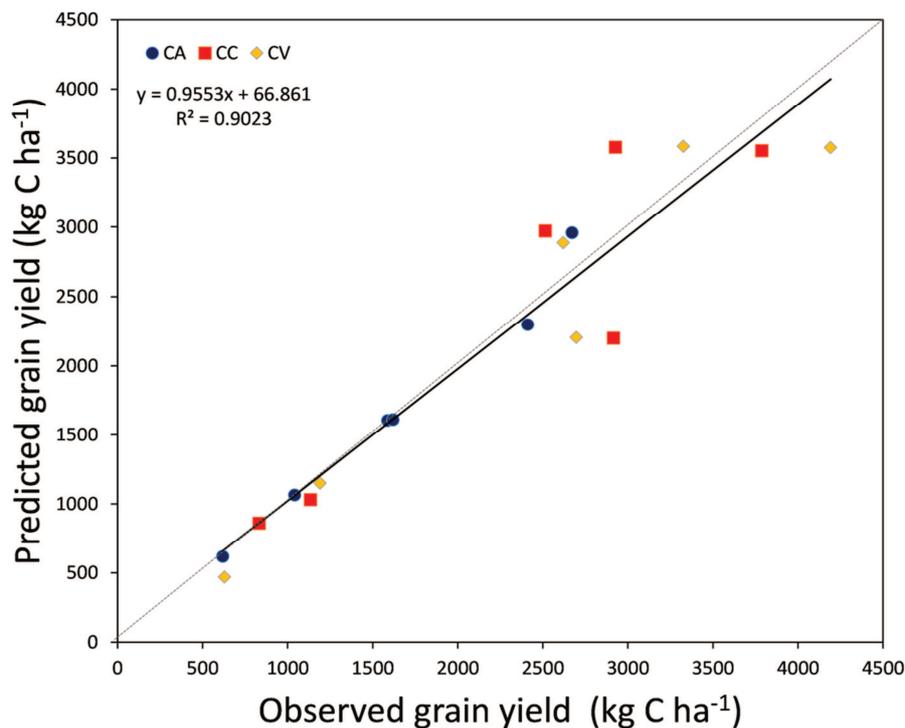


Figure 4 Comparison of observed and predicted grain yield (kg C ha<sup>-1</sup>) by DNDC model during 2011-2017.

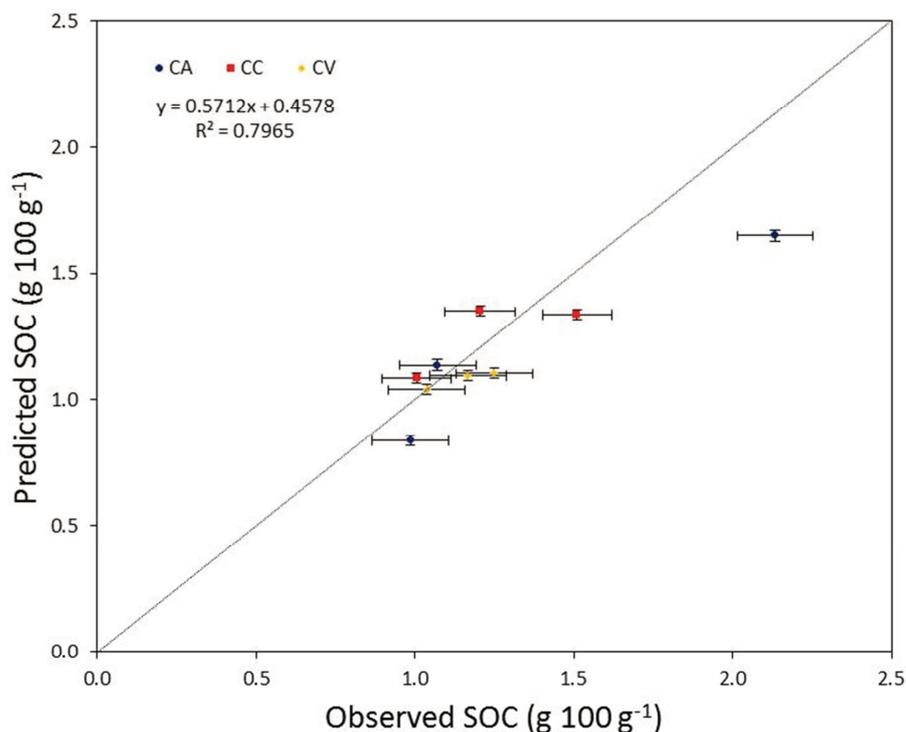


Figure 5 Comparison of observed and predicted SOC concentrations (g 100 g<sup>-1</sup>) by DNDC model between 2016-2017 as a result of Monte Carlo simulations. Error bars represent standard error.

## 2.6. DNDC scenario analyses

Long-term soil organic carbon stock dynamics and gas emissions (2018-2122) were assessed with the DNDC model in CA, CC, and CV treatments by taking into account climate change scenarios provided by the Intergovernmental Panel on Climate Change (IPCC, 2007). The IPCC (Nakicenovic *et al.*, 2000) has outlined scenarios of increasing atmospheric carbon dioxide (CO<sub>2</sub>) concentrations and their evolution in the 21<sup>st</sup> century. In this study, the A1B (“Rich world”), A2 (“Separated world”), and B1 (“Sustainable world”) scenarios were selected, each of which was characterised by a different CO<sub>2</sub> concentration (Table 2). The scenarios underwent some simplifications: a) climate change were narrowly defined as only rainfall and air temperature variations, which neglected the potential effects from increased CO<sub>2</sub> on other factors, such as biomass yield; b) only climate data with no socio-cultural or economic predicted change was considered; c) potential adaptations of farm management systems (e.g., selection of new crop species and varieties, application of efficient irrigation methods) to climate change scenarios were not considered; d) IPCC Special Report on Emission Scenarios (Nakicenovic *et al.*, 2000), instead of the most recent IPCC Representative Concentration Pathways (IPCC, 2013), was used for consistency and comparison with previous studies conducted in Veneto (Dal Ferro *et al.*, 2018).

The climate conditions of the low Venetian plain were modelled using LARS-WG v.5.0 software, a stochastic data generator based on models provided by the IPCC (Semenov *et al.*, 2013). Weather parameters were calibrated with locally observed daily weather variables. Climatic projections, provided by the model were generated at fixed CO<sub>2</sub> concentrations for three subsequent periods (Table 2) with static temperature and rainfall data. We used the “IPCM4” model developed by Hourdin *et al.* (2006) that provides daily temperature and precipitation for each scenario.

Scenario	Key assumptions	CO <sub>2</sub> concentration (ppm)		
		2018–2045	2046–2080	2081–2122
A1B “The rich world”	Characterised by very rapid economic growth (3% yr <sup>-1</sup> ), low population growth (0.27% yr <sup>-1</sup> ) and rapid introduction of new and more efficient technology. Globally there is economic and cultural convergence and capacity building, with a substantial reduction in regional differences in per capita income.	418	541	674
A2 “The separated world”	Cultural identities separate the different regions, making the world more heterogeneous and international cooperation less likely. “Family values”, local traditions and high population growth (0.83% yr <sup>-1</sup> ) are emphasised. Less focus on economic growth (1.65% yr <sup>-1</sup> ) and material wealth.	414	545	754
B1 “The sustainable world”	Rapid change in economic structures, “dematerialization” including improved equity and environmental concern. There is a global concern regarding environmental and social sustainability and more effort in introducing clean technologies. The global population reaches 7 billion by 2100.	410	492	538

Table 2 CO<sub>2</sub> concentrations for selected climate scenarios specified in the Special Report on Emissions Scenarios (Nakicenovic *et al.*, 2000; Semenov *et al.*, 2013).

## 2.7. Statistical analysis

Crop yield and biomass, water percolation and upflux, SOC and TN concentrations, and SOC stock variations (between 2017 and 2011) were analysed with linear mixed-effect modelling based on REML (Restricted Maximum Likelihood) estimation. It considered clay and sand contents as continuous factors and treatment and soil layer (only for SOC and TN concentration) as categorical factors. SOC stock variation was tested for each treatment by increasing soil profiles (0-5 cm, 0-30 cm, and 0-50 cm). Data from each treatment of the same field were considered as sub-replicates and treated as nested measures. All possible first order interactions between factors were tested and the model with the smallest AIC (Akaike’s Information Criterion) was selected (Schabenberger and Pierce, 2002). According to the Shapiro–Wilk normality test, some variables (leached water and

upflux) were log-transformed before the analysis to improve the normal distribution assumption. The Kruskal–Wallis test was used for non-parametric water quality data (NO<sub>3</sub>-N concentration in lysimeters and groundwater) across different treatments. Post-hoc pair-wise comparisons of least-squares means were performed using the Tukey method to adjust for multiple comparisons. Statistical analyses were performed with SAS software (SAS Institute, Inc., Cary, NC, USA) version 6.1 and STATISTICA software (Stat Soft, Inc., Tulsa, OK, USA) version 8.0.

### 3. Results

#### 3.1. Weather and soil monitoring

Contrasts were observed between the two years of weather data (March-December 2016; January-August 2017). While rainfall events were evenly distributed during the spring and autumn in both years, total amounts differed. Total rainfall during April - June 2016 was 278 mm; the same period in 2017 totalled far less (about 123 mm). Similar differences were observed during July - August (87 mm in 2016; 45 mm in 2017). As expected, groundwater levels responded with a generalised fall in the summer period and attained a minimum of about -250 cm. Also as expected, the level rose ( $> -100$  cm) in late autumn-winter and after heavy rainfalls (Figure 6). Among treatments, variations were negligible except in the 2016 April - July monitoring period when the water table was higher in CV than in CC and CA. In summer 2017, the opposite condition was observed.

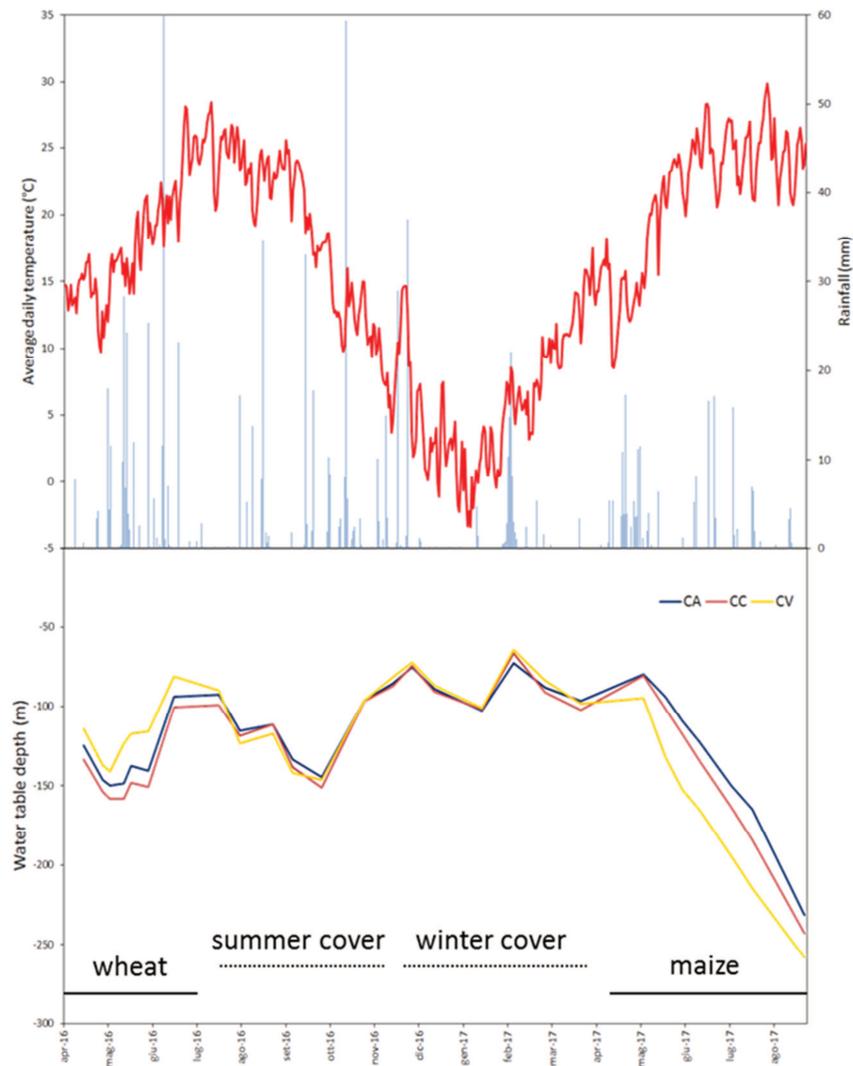


Figure 6 Average daily temperature, rainfall, and groundwater table dynamics in the monitored fields (April 2016-August 2017). Cropping (CA, CC, CV) and intercropping seasons (only CA and CC) are represented by solid and dashed lines, respectively.

Both field treatments and weather conditions affected soil-water content, especially in the topsoil layer. For example, CA produced higher content values than did CV in the 0-30 cm soil profile (Figure 7a) throughout the monitoring period. In particular, larger differences — as much as  $0.15 \text{ m}^3 \text{ m}^{-3}$  on June 29, 2017 — were found after ploughing operations. Apparently, the effects of tillage differences persisted until the end of the monitoring period. Conversely, CV water content was higher than in CA at a depth of -55 cm, where the above-mentioned trend was markedly reversed.

Soil moisture differences between CC and CV (Figure 7b) were found mainly at the soil surface (10 cm depth) during both spring and summer; on average, CV was  $0.03 \text{ m}^3 \text{ m}^{-3}$  higher than CC. By contrast, soil moisture in CC during autumn–winter was slightly higher than in CV, a difference that was pronounced after November 2016 tillage operations. At the 55 cm soil depth, moisture levels were similar between the two treatments and throughout the monitoring period.

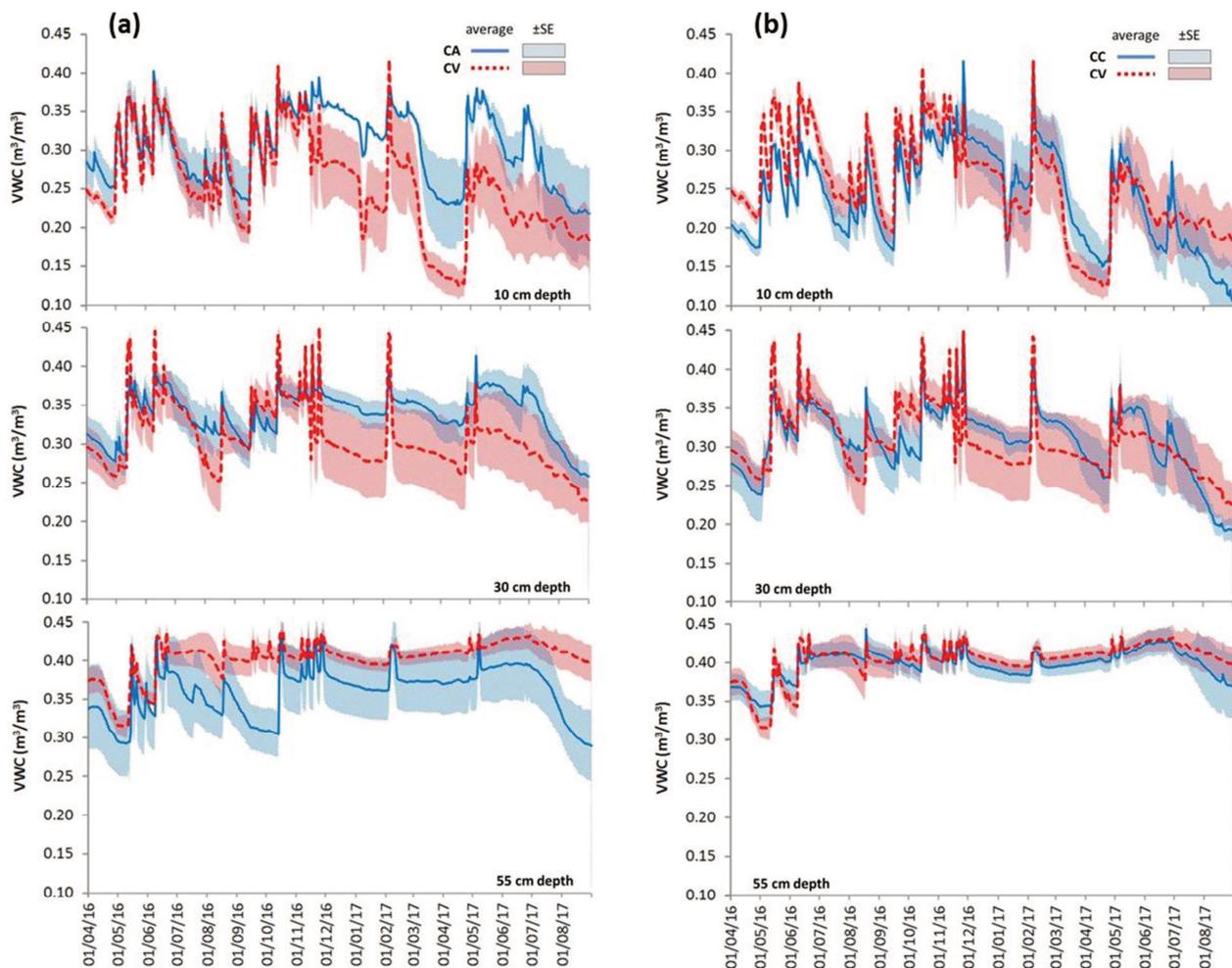


Figure 7 Average soil moisture (solid and dashed line) and standard error (coloured area) at different depths during the period April 2016–August 2017. Comparison of CA (a) and CC (b) with CV.

Soil temperature varied little across the monitoring stations, regardless of CV, CA, or CC practice differences. Higher temperatures were noted at all depths (+0.8 °C, on average) in CA than in CV and CC until summer 2016 ended. As September 2016 began, all treatments exhibited negligible differences through June 2017; then CV was an average 1.4 °C above CA and CC (data not shown).

### 3.2. Biomass and crop yield production

Overall, total biomass production (main crops and cover crops) throughout the seasons of the two-year monitoring period did not differ significantly across the treatments. In rank order, CC had a slightly higher production (32.5 Mg ha<sup>-1</sup>) than did CV (29.3 Mg ha<sup>-1</sup>), and CA ranked lowest (24.7 Mg ha<sup>-1</sup>) (Table 3).

Treatments	Year	Cultivated plants	Crop yield (Mg ha <sup>-1</sup> )	Crop residues (Mg ha <sup>-1</sup> )	Total aboveground biomass (Mg ha <sup>-1</sup> )
CA	2016	Winter wheat	6.68 (±0.03)	6.25 (±0.16)	12.93 (±0.19)
		Summer cover crop	-	-	2.52 (±0.31)
	2017	Winter cover crop	-	-	0.31 (±0.07)
		Maize	4.05 (±0.82)	4.85 (±0.62)	8.90 (±1.26)
	Total		10.73 (±0.97)	11.10 (±0.67)	24.66 (±1.55)
CC	2016	Winter wheat	6.29 (±0.15)	6.12 (±0.25)	12.41 (±0.41)
		Summer cover crop	-	-	4.88 (±0.25)
	2017	Winter cover crop	-	-	n.a.
		Maize	9.47 (±0.37)	5.77 (±0.74)	15.24 (±0.70)
	Total		15.76 (±0.65)	11.89 (±0.76)	32.53 (±1.07)
CV	2016	Winter wheat	6.55 (±0.07)	6.31 (±0.12)	12.86 (±0.16)
	2017	Maize	10.48 (±0.45)	5.99 (±0.76)	16.47 (±0.87)

Table 3 Aboveground biomass production of the main crops (grain yield residues) and cover crops during the monitoring period (standard error in brackets).

Winter wheat yields did not differ among treatments. Grain yields averaged 6.50 Mg ha<sup>-1</sup>, and ranged between a minimum of 6.29 Mg ha<sup>-1</sup> in CC and a maximum of 6.68 Mg ha<sup>-1</sup> in CA. Average aboveground crop wheat biomass with residues was 12.73 Mg ha<sup>-1</sup>, with similar range trends and

sizes between 12.41 Mg ha<sup>-1</sup> (minimum) in CC and 12.93 Mg ha<sup>-1</sup> (maximum) in CA. Significant differences ( $p < 0.05$ ) were observed during spring-summer 2017, when the CA maize yield was 40% (4.05 Mg ha<sup>-1</sup>) below CC and CV (9.97 Mg ha<sup>-1</sup>, on average). Maize aboveground crop biomass with residues was also significantly higher in CC and CV (15.86 Mg ha<sup>-1</sup>, on average) than in CA (8.9 Mg ha<sup>-1</sup>).

Both CA and CC treatments resulted in low aboveground biomass values for the summer cover crop (sorghum - sudangrass), averaging 3.7 Mg ha<sup>-1</sup>. A significant difference ( $p < 0.01$ ) in biomass production was found between CA and CC (2.52 and 4.88 Mg ha<sup>-1</sup>, respectively). The negligible winter cover crop production in CC was notable and in contrast to that of CA (0.31 Mg ha<sup>-1</sup>), where a sowing delay due to climatic and management constraints dramatically reduced germination and seedling growth in both systems.

### 3.3. Water balance

During April to December 2016, precipitation totalled 637 mm and exceeded average crop ET by 100 mm (Table 4). During January - August 2017 precipitation was more than half (55%) the ET (286 mm *versus* 520 mm, respectively). A comparison between different years (an evaluation was possible for the only April-August period) highlighted that precipitation was 80% (2016) and 40% (2017) of average crop ET, respectively, that was likely the result of both different weather conditions and crop-specific water requirements.

This result highlighted the high water demand of maize (crop season 2017), especially in CV during the summer when ET quadrupled the rainfall (360 mm and 96 mm, respectively).

The different ET values found in the treatments also affected percolation depth. During 2016, continuously covered soil systems (CA and CC) demonstrated comparatively lower percolation (178 mm, on average) than CV (283 mm), whereas in 2017, values of percolation of all treatments fell (78.6 mm, on average). In contrast, upflux showed the inverse trend (significantly higher in CV than CA and CC), with lower values in 2016 than in 2017 (Table 4). In 2017, sub-surface water partially compensated for the crop and cover crop water demand, which reversed the 2016 net positive percolation.

Treatments	Year	Rainfall (mm)	ET (mm)	Percolation (mm)	Upflux (mm)	Net percolation <sup>a</sup> (mm)
CA	2016	637	572	178.6 (±6.8)	139.9 (±4.6)	38.7 (±7.9)
	2017	286	510	67.5 (±6.7)	232.7 (±13.9)	-165.2 (±20.2)
CC	2016	637	598	177.7 (±9.8)	169.0 (±3.9)	8.6 (±8.0)
	2017	286	462	87.0 (±11.5)	191.8 (±3.1)	-104.8 (±8.4)
CV	2016	637	441	283.0 (±25.2)	93.0 (±9.3)	190.0 (±23.7)
	2017	286	589	81.2 (±2.9)	353.9 (±7.1)	-272.8 (±9.7)

<sup>a</sup> Net percolation: difference between Percolation and Upflux.

Table 4 Water balance in the monitored fields during the periods April-December 2016 and January-August 2017 (standard error in brackets).

### 3.4. Water quality

Suction lysimeter  $\text{NO}_3\text{-N}$  concentrations differed between 2016 (Figure 8a) and 2017 (Figure 8c). During the 2016 cropping season and until March 2017, all treatment values measured less than  $60 \text{ mg l}^{-1}$ , and then increased markedly during summer 2017 until they peaked above  $200 \text{ mg l}^{-1}$ . Among treatments,  $\text{NO}_3\text{-N}$  was lower in CA than in CC and CV in both years.

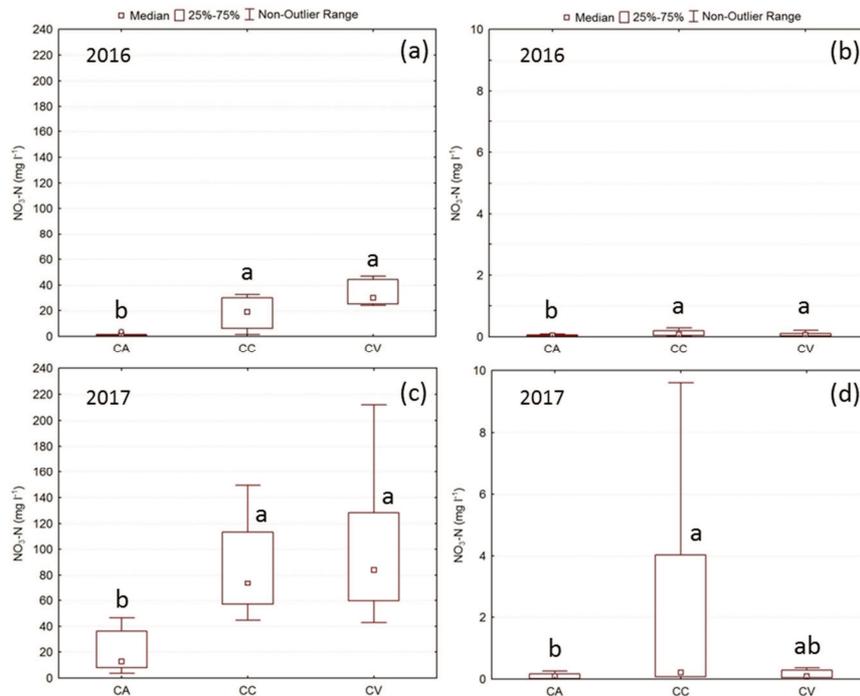


Figure 8 Nitrate concentrations from suction lysimeters (a, c) and water table wells (b, d) during 2016 (April-December 2016) and 2017 (January-August 2017). Note the different y-axis scale in (a, c) versus (b, d). Values differ significantly when labelled with with different letters (Tukey post hoc test with  $p \leq 0.05$ ).

Observed 2016 groundwater values were different across the treatments, with significantly lower values in CA as opposed to CC and CV (Figure 8b). However, groundwater NO<sub>3</sub>-N concentrations, collected in the phreatic wells, were much lower than those found in the suction lysimeters.

### 3.5. Nitrogen balance

Nitrogen (N) fertilisation and rainfall inputs were identical among the treatments; therefore, N balance changes mainly stemmed from plant uptake ( $N_{\text{uptake}}$ ), N leaching ( $N_{\text{leach}}$ ), and upflux ( $N_{\text{up}}$ ) (Table 5). Conservation agriculture (CA) showed the lowest  $N_{\text{net leaching}}$  ( $N_{\text{leach}} - N_{\text{up}}$ ) in both 2016 and 2017, resulting from very low NO<sub>3</sub>-N water concentrations leaving the 0-60 cm layer (3.5 kg ha<sup>-1</sup>, on average). Continuous soil cover with cover crops (CC) also restricted N leaching with respect to CV in 2016. 2017 results of CC were similar to those in CV as the sparse cover crops failed to catch N losses adequately. The N balances over the experimental period indicated an average annual surplus in CA and CC of 80.4 and 10.2 kg N ha<sup>-1</sup>, respectively, and a deficit in CV of -20.6 kg N ha<sup>-1</sup>.

Treatments	Year	$N_{\text{fert}}$ (kg ha <sup>-1</sup> )	$N_{\text{rain}}$ (kg ha <sup>-1</sup> )	$N_{\text{uptake}}$ (kg ha <sup>-1</sup> )	$N_{\text{net leaching}}^{\text{a}}$ (kg ha <sup>-1</sup> )	$N_{\text{bal}}$ (kg ha <sup>-1</sup> )
CA	2016	146	7.6	140.3	3.0	10.3
	2017	212	3.4	61.0	4.0	150.4
CC	2016	146	7.6	132.1	31.2	-9.7
	2017	212	3.4	142.0	43.4	30.0
CV	2016	146	7.6	137.6	66.3	-50.3
	2017	212	3.4	157.0	49.2	9.2

<sup>a</sup>  $N_{\text{net leaching}}$ : difference between nitrogen from leaching ( $N_{\text{leach}}$ ) and upflux ( $N_{\text{up}}$ ) (Eq. 4).

Table 5 Nitrogen balance in the monitored fields during 2016 (April-December) and 2017 (January-August).

### 3.6. Soil organic carbon and total nitrogen concentrations

Soil organic carbon (SOC) concentrations in 2017 differed significantly ( $p < 0.01$ ) by treatment, depth, and clay content. Along the soil profile (0-50 cm), SOC averaged 1.20 g<sup>-1</sup> 100 g<sup>-1</sup> in CA, 1.13 g<sup>-1</sup> 100 g<sup>-1</sup> in CC, and 1.10 g<sup>-1</sup> 100 g<sup>-1</sup> in CV. The 0–5 cm soil layer influenced SOC averages most as borne out by significantly higher values in CA (2.20 g<sup>-1</sup> 100 g<sup>-1</sup>) relative to CC (1.50 g<sup>-1</sup> 100 g<sup>-1</sup>) and CV (1.16 g<sup>-1</sup> 100 g<sup>-1</sup>) (Figure 9). At depths below 5 cm, no differences were observed

among the treatments. Total nitrogen (TN) differentiated CA ( $0.31 \text{ g}^{-1} 100 \text{ g}^{-1}$ ) from CC and CV (both  $0.22 \text{ g}^{-1} 100 \text{ g}^{-1}$ ) ( $p < 0.01$ ) in the shallow layer (0-5 cm) (Figure 9), whereas clay content significantly affected TN over the entire 0-50 cm profile ( $p < 0.01$ ). Finally, C/N ratio differentiated both CA and CC (6.96, on average) from CV (5.27) (Figure 9).

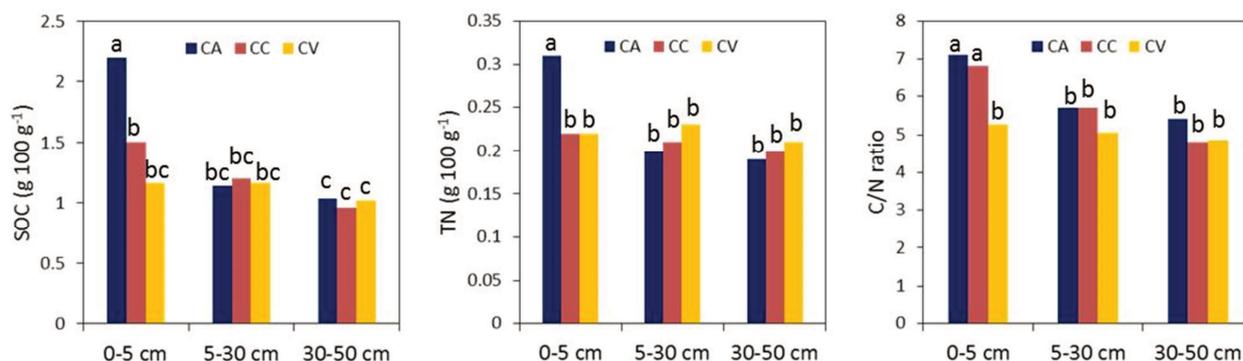


Figure 9 Soil organic carbon (SOC) concentration (left), total nitrogen (TN) concentration (centre), and C/N ratio (right) at different soil layers in CA, CC, and CV treatments. Values differ significantly when labelled with different letters (Tukey post hoc test with  $p \leq 0.05$ ).

Differences between 2017 and 2011 in SOC stocks were tested in the 0–5 cm, 0–30 cm, and 0–50 cm soil profiles using the equivalent soil mass method (Figure 10). Initial SOC stocks in 2011 were  $5.1 \text{ Mg C ha}^{-1}$  at 0-5 cm,  $29.7 \text{ Mg C ha}^{-1}$  at 0-30 cm and  $57.4 \text{ Mg C ha}^{-1}$  at 0-50 cm, on average, with no significant treatment differences. Significant differences ( $p < 0.01$ ) were found in the top layer (0-5 cm), where CA increased  $4.4 \text{ Mg C ha}^{-1}$ , CV rose slightly ( $0.3 \text{ Mg C ha}^{-1}$ ), and CC fell slightly ( $-0.4 \text{ Mg C ha}^{-1}$ ). Deep soil layer SOC stock analysis and quantification showed consistent accumulation ( $6.4 \text{ Mg C ha}^{-1}$  at 0-30 cm and  $10.5 \text{ Mg C ha}^{-1}$  at 0-50 cm, on average) over the six-year period, with no significant treatment differences.

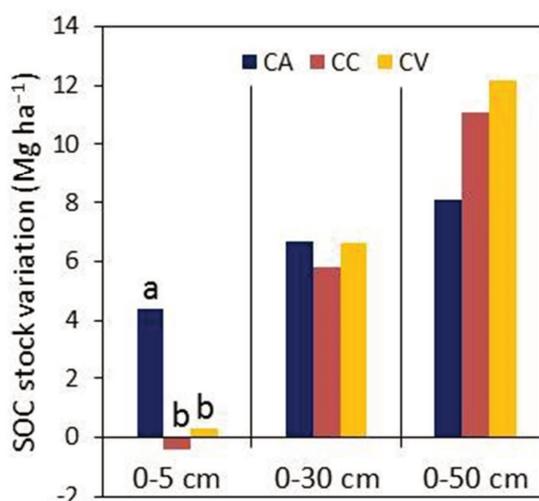


Figure 10 Soil organic carbon (SOC) stock variations from 2011 to 2017. Values differ significantly when labelled with different letters (Tukey post hoc test with  $p \leq 0.05$ ).

### 3.7. DNDC model prediction of gas emissions

Nitrous oxide emissions, predicted with the DNDC model, were generally lower in CC and CA than in CV during both the 2016 and 2017 cropping seasons (Table 6). Specifically, CA exhibited the lowest N<sub>2</sub>O fluxes during the winter wheat cropping season (1.60 kg N ha<sup>-1</sup>), followed by CC (1.81 kg N ha<sup>-1</sup>), and CV (3.71 kg N ha<sup>-1</sup>). In 2017, emissions were also highest in CV (5.89 kg N ha<sup>-1</sup>) as opposed to similar values in CC and CA despite the fact that overall average values were more than doubled in 2017 than in 2016, as a result of meteorological and agronomic factors (e.g., fertiliser input). Alternatively, ammonia emissions were higher in CA than in CC and CV (46.5 kg N ha<sup>-1</sup> *versus* an average of 36.3 kg N ha<sup>-1</sup>) and were similar in both 2016 and 2017 (Table 6).

In the case of N<sub>2</sub> emissions, they too were higher in CA relative to CC and CV (+22% and +5% on average, respectively). Methane (CH<sub>4</sub>) emission results provided by the model indicated no net emissions; values were -0.70 and -0.63 kg N ha<sup>-1</sup> y<sup>-1</sup> with no significant treatment difference.

Net CO<sub>2</sub> (difference between efflux and influx) in 2016 was similar in CA and CC (-2526.9 kg C ha<sup>-1</sup>, on average), and significantly higher in CV (-1583.6 kg C ha<sup>-1</sup>). In 2017, CC continued to show negative net CO<sub>2</sub> (influx higher than efflux), while CA and CV produced a low positive value.

Treatments	Year	N <sub>2</sub> O (kg N ha <sup>-1</sup> )	NH <sub>3</sub> (kg N ha <sup>-1</sup> )	N <sub>2</sub> (kg N ha <sup>-1</sup> )	CH <sub>4</sub> (kg C ha <sup>-1</sup> )	Net CO <sub>2</sub> <sup>a</sup> (kg C ha <sup>-1</sup> )
CA	2016	1.96 (±0.03)	24.00 (±0.04)	115.22 (±18.24)	-0.78 (±0.004)	-2118.7(±5.6)
	2017	4.24 (±0.04)	69.54 (±0.05)	169.46 (±17.47)	-0.90 (±0.005)	75.3 (±4.9)
CC	2016	2.42 (±0.04)	17.29 (±0.02)	109.52 (±21.06)	-0.79 (±0.004)	-2085.0 (±5.0)
	2017	3.83 (±0.04)	55.35 (±0.03)	106.81 (±21.77)	-0.93 (±0.004)	-1322.3 (±9.3)
CV	2016	4.10 (±0.07)	18.01 (±0.03)	113.56 (±22.29)	-0.70 (±0.004)	-1309.0 (±4.8)
	2017	6.04 (±0.06)	55.52 (±0.03)	141.26 (±26.19)	-0.81 (±0.005)	-951.2 (±7.8)

<sup>a</sup> Net CO<sub>2</sub>: difference between efflux and influx C.

Table 6 Gas emissions from the monitored fields during 2016-2017. Data from DNDC model (standard error in brackets).

### 3.8. DNDC scenario results

Weather modelled with LARS-WG predicted a maximum deviation of 3.7 °C from actual average annual temperatures for subsequent periods. Larger differences were observed in the last modelled period (2018-2122), which corresponded to the largest estimated atmospheric CO<sub>2</sub> concentrations.

As for precipitation predictions, no significant differences were found among the scenarios; the annual average was higher in B1 (731 mm) than in A1B (725 mm) and A2 (699 mm).

SOC stocks varied principally with land management practices, while climate scenarios, represented within each treatment between maximum-minimum range (Figure 11), had more minor effects during the entire simulation period. The SOC stock in CA never attained stability, which confirmed the existence of a high potential to sequester SOC. Moreover, the similar SOC accumulation in CV and CA for 17 years after initial simulation (*ca.* 2035) demonstrated the dynamic of slow SOC accumulation under untilled soil conditions in the 0-50 cm layer.

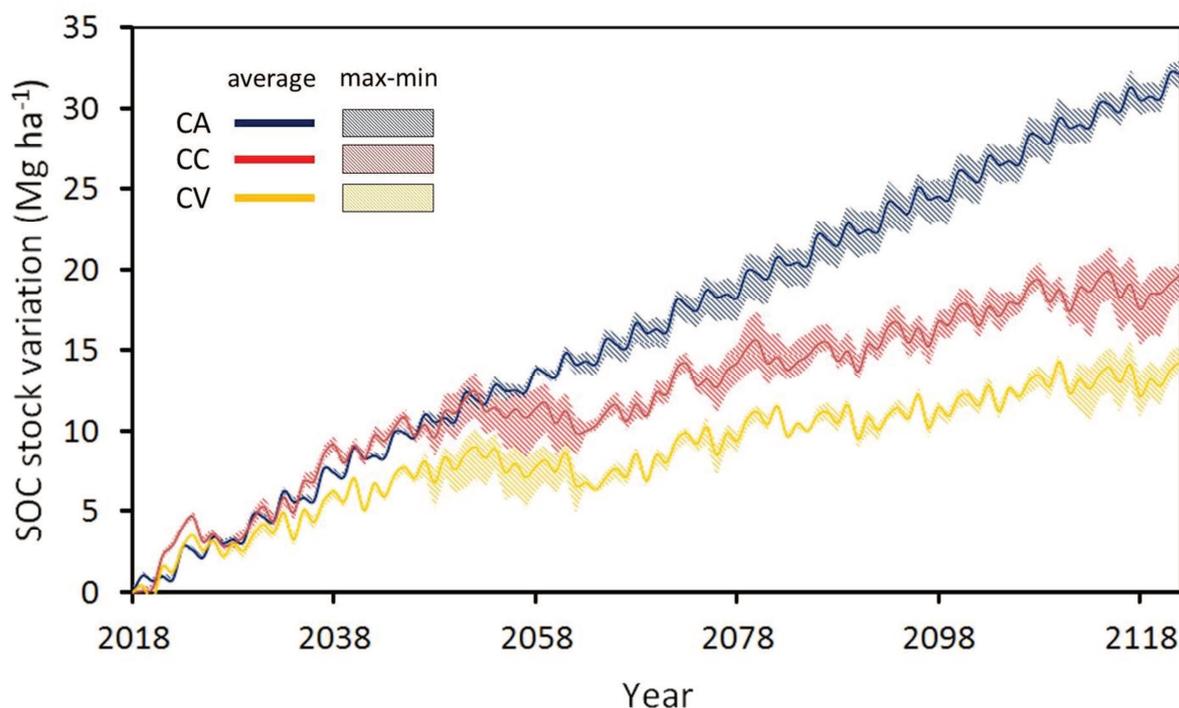


Figure 11 Trend of average SOC stock variation (solid line) during the period 2018- 2122 (modelled data from DNDC simulation). Maximum-minimum values (coloured area) were predicted from different climate scenarios (B1, A1B, A2) (Nakicenovic *et al.*, 2000).

In the case of N<sub>2</sub>O emissions, the model predicted lower values in CA than in CC and CV for the first period, followed by a long period of trend reversal. Over the 105-year simulation, CA emitted 50% more N<sub>2</sub>O each year than did CC and CV (Table 7). In terms of global warming potential (GWP, 1 CO<sub>2</sub>: N<sub>2</sub>O = 265; CH<sub>4</sub> = 28) (IPCC, 2013), higher SOC accumulations failed to offset this N<sub>2</sub>O emission increase, as evidenced by 44% greater net GWP values in CA than in the very similar results in CC and CV (Table 7).

Scenarios	Treatments	CO <sub>2</sub>	N <sub>2</sub> O	CH <sub>4</sub>	Net GWP <sup>a</sup>
		Avg. flux rate (kg C ha <sup>-1</sup> yr <sup>-1</sup> )	Avg. flux rate (kg N ha <sup>-1</sup> yr <sup>-1</sup> )	Avg. flux rate (kg C ha <sup>-1</sup> yr <sup>-1</sup> )	(kg eq.CO <sub>2</sub> ha <sup>-1</sup> yr <sup>-1</sup> )
A1B	CA	-314.9 (±84.8)	6.9 (±0.3)	-1.1 (±0.02)	1679.8 (±436.0)
	CC	-228.3 (±96.3)	4.8 (±0.2)	-1.0 (±0.02)	1126.0 (±436.5)
	CV	-160.2 (±107.3)	4.5 (±0.2)	-0.9 (±0.01)	1254.0 (±476.4)
A2	CA	-300.0 (±81.6)	7.0 (±0.4)	-1.1 (±0.02)	1775.9 (±466.0)
	CC	-207.5 (±101.0)	4.8 (±0.2)	-1.1 (±0.02)	1198.4 (±453.7)
	CV	-158.2 (±114.9)	4.7 (±0.2)	-1.0 (±0.01)	1340.9 (±504.2)
B1	CA	-309.1 (±87.5)	7.3 (±0.4)	-1.1 (±0.02)	1867.6 (±487.6)
	CC	-228.0 (±98.7)	5.0 (±0.2)	-1.0 (±0.01)	1210.3 (±444.9)
	CV	-172.9 (±112.3)	4.7 (±0.2)	-0.9 (±0.01)	1290.8 (±494.7)

<sup>a</sup> GWP values relative to CO<sub>2</sub>: N<sub>2</sub>O = 265; CH<sub>4</sub> = 28.

Table 7 Greenhouse gas emissions (positive values) or uptake (negative values) during long-term simulation (2018-2122) (standard error in brackets).

## 4. Discussion

Crop production was similar among the various treatments during winter-wheat cultivation; however, significant differences in maize production were observed during the spring-summer season, with lower grain yields in CA than in CC and CV. This result suggests that the adoption of no tillage was the primary factor to influence crop production, likely due to seed germination and plant growth difficulties (Constantin *et al.*, 2010). Low crop production in no-till systems has already been reported in other studies, especially during transition phases with yield recovery periods lasting up to 10 years (Rusinamhodzi *et al.*, 2011; Pittelkow *et al.*, 2015). At the same time, others (e.g., Palm *et al.*, 2014) found no differences under low fertility and rain-fed conditions. In this case, it was hypothesised that the combination of continuous soil cover and undisturbed soil would favour soil moisture retention and result in higher, more stable yields in CA during the dry seasons.

Although our field experiment was managed under rain-fed conditions, the water table contributed amply to crop water demand, especially in 2017 when evapotranspiration largely exceeded precipitation. Indeed, the contribution of upflux on water balance was relevant, ranging between 21% and 28% of ET in 2016 and between 42% and 60% in 2017. Extreme drought conditions of August 2017 sharply reduced the soil moisture and, therefore, evapotranspiration, especially in CC. However, the low soil moisture in August only slightly affected value of total biomass because most of maize water need was in July during flowering and grain setting (Allen *et al.*, 1998). In CA, water balance was also most likely affected by poor soil structure. Indeed, compacted soil slowed water infiltration and redistribution within the profile, resulting in higher water content in the upper layers and lower content in the deeper layers compared to CV. This contrasts with the review by Soane *et al.* (2012) that put forth infiltration increased under no-till conditions as a result of improved soil structure conditions (more vertically-oriented macro-porosity and pore continuity). However, previous experiments on similar silty soils showed CA was slow to improve soil structure characteristics due to its low organic carbon content (Piccoli *et al.*, 2017).

Despite drought conditions in summer 2017, negligible differences in soil moisture were caused by cover crop cultivation. Most likely, poor cover crop growth mitigated water competition, such that cash crop development was unaffected. Nevertheless, factors such as cover crop cultivation duration and growth may affect soil moisture (Pinto *et al.*, 2017), making careful management essential in order to optimise biomass production and hydrologic regulation ecosystem services.

In our case, poor cover crop growth resulted from a non-optimal planting time due to narrow windows for their cultivation.

The primary goal of intercropping with cover crops is to mediate root zone nutrient leaching (Thorup-Kristensen, 2003). However, this goal was only partially accomplished due to low crop growth. Both soil solution and groundwater  $\text{NO}_3\text{-N}$  concentrations in 2016 and 2017 were significantly lower only when no tillage was practiced. Verhulst *et al.* (2010) already noticed that no tillage with residual retention was associated with a higher N immobilisation by remaining the soil surface residues, which reduced the potential for N losses. However, it must be noted that groundwater  $\text{NO}_3\text{-N}$  concentration was very low in all systems, and in general, did not exceed EU drinking water limits ( $11 \text{ mg l}^{-1}$ ) over the entire monitoring period. In this context, the remarkable upflux directly affected groundwater quality by reducing N leaching. Most probably, the combination of upward movement, dilution, and denitrification were the primary processes that limited groundwater  $\text{NO}_3\text{-N}$  concentrations (Weil *et al.*, 1990; Morari *et al.*, 2012). Indirectly, the relevance of the denitrification process was confirmed by DNDC even if the model was not validated for gas emissions.

Conservation agriculture and cover crops do not regulate atmospheric greenhouse gas emissions in a straightforward way. For instance, both higher (Rochette *et al.*, 2008) and lower (Gregorich *et al.*, 2008)  $\text{N}_2\text{O}$  losses were measured at times in no tillage and in tillage treatments. Model simulations also predicted  $\text{N}_2\text{O}$  emissions might be positive (Li *et al.*, 2005) or negative (Li *et al.*, 1996), depending on pedo-climatic and management conditions. According to prior research by Mutegi *et al.* (2010), our results indicate that incorporated crop residues in CC and CV resulted in significantly higher emissions than produced in CA. Alternatively, CA emitted at the following levels, on average: 1) higher levels of  $\text{NH}_3$  (+26.6%) than did CV, attributed to the lack of incorporated urea (Patra *et al.*, 2004); 2) higher levels of  $\text{N}_2$ , attributed to wetter topsoil conditions that favoured the chemical reduction of  $\text{N}_2\text{O}$  emissions to  $\text{N}_2$  (+5.3% in CA *versus* CV, on average) (Soane *et al.*, 2012). These results are consistent with those obtained by solving the N balance, and might explain the significant decrease of N leaching in CA, despite the overall low N uptake by biomass production. Indeed, CA was the system with a higher residual term  $N_{bal}$ , which represents a combined term that includes N air losses (volatilisation and denitrification) and the change in N content of the soil profile between the beginning and end of the monitored N balance period. Since results in soil experimental N content did not differ significantly between the LM systems, the higher values of the residual term  $N_{bal}$  in CA could be associated with higher gaseous losses than CC and CV, as predicted by DNDC. Finally, it must be noted that LM systems were all

characterised by higher N gas emissions than the residual term  $N_{bal}$ , suggesting overall N losses in CA, CC and CV treatments during the two-year monitoring period.

An average reduction in N<sub>2</sub>O emissions of 50%, relative to the conventional system, from mitigation efforts was predicted in CA. However, our model application found few robust and confirmatory studies on N<sub>2</sub>O, and even fewer field experiments on N<sub>2</sub> fluxes, a situation that makes evident the need for more research on N atmospheric fluxes.

In the long term, N<sub>2</sub>O results reversed markedly with higher emissions in CA than in CV and CC, which confirmed the high level of uncertainty associated with GHG studies and the need for long-term monitoring experiments (Knapp *et al.*, 2012).

During the monitoring period, DNDC simulated negative net CO<sub>2</sub> emissions, suggesting that all agricultural systems were already accumulating SOC in the span of just two years (700 kg C ha<sup>-1</sup>, on average). However, fluctuations of net CO<sub>2</sub> fluxes occurred when longer simulation periods included both inter-annual weather variability and the entire three-year crop rotation. SOC stock dynamics, predicted with DNDC model in 2016-2017, agreed with experimental results, and highlighted that differences among treatments were not found in the 0-30 cm soil layer or at greater depths (down to 50 cm) (Figure 4). As already observed by other authors (Luo *et al.*, 2010; Powlson *et al.*, 2011; Piccoli *et al.*, 2016), SOC was stratified differently in the no tillage treatment (CA) only, and not in the tillage (CV and CC) treatments. This result suggests that CA proposed practice to increase carbon sequestration might not produce improvement. In this context, it should be highlighted that some underestimation in SOC prediction was observed with DNDC model in the 0-5 cm (Figure 4). Nevertheless, differences between modelled and experimental data observed in the surface layer (0-5 cm) only partially affect the overall SOC stock estimation within the soil profile (0-50 cm).

Retention of crop residues on the soil surface and the absence of tillage operations drove SOC dynamics in the topsoil layer of CA, while residue incorporation with ploughing was responsible for SOC accumulation in the deeper layers in CV and CC (Govaerts *et al.*, 2009). However, different SOC stock dynamics in the long term should not be ignored. Alvarez (2005), for example, found that SOC accumulated under a no tillage treatment following an S-shaped, time-dependent process that reached steady state after 25-30 years. Moreover they observed that in the short term (10 years of experimentation) accumulation was negligible. Similarly, long-term DNDC modelling showed that SOC accumulation dynamics along the soil profile (especially in CC and CV) attained a quasi-steady state. On the other hand, the low reactivity of silty soils in Veneto to conservation agriculture

(Piccoli *et al.*, 2017) was demonstrated as SOC in CA was far from stabilised conditions, even after 100 years of simulation. In spite of its higher SOC sequestration, CA does not seem a win-win solution for GHG mitigation in the long run, at least considering predictions at the field scale. Indeed, net GWP model predictions of GHGs were 44% higher in CA than CC and CV because of increased N<sub>2</sub>O emissions. Note that an overall evaluation of CA impact would imply to conduct a Life Cycle Assessment beyond the farmgate.

Eventually, a minor role was attributed to alternative long-term climate variability since changing scenarios resulted in trends similar to those observed in previous studies conducted at the European scale (Lugato *et al.*, 2014).

## 5. Conclusion

The evaluation of two land management practices in Veneto Region confirmed the lack of a “perfect” management, that is, one capable of improving soil functions and providing consistent ecosystem services. Indeed, land management practices may provide biomass production, but not regulate the water quality simultaneously or *vice versa*. In the latter case, the same practice may deliver contrasting effects on water quality and climate change mitigation. Furthermore, a number of ecosystem services provided in the short and medium terms may even be reversed over the long term. Above all, high levels of uncertainty can be introduced by variable pedo-climatic conditions and their interaction with the soil management.

In our experiment, conservation agriculture and cover crops results contrasted according to the soil functions, the ecosystem service category and evaluation time span. The former was more effective in providing regulating services in the short term, and less consistent in the long term, at least for GHG mitigation. GHG control is only one of the numerous ecosystem services provided by conservation practices (e.g. reduction of erosion and P particulate loss). Many of these depend on the C content which are strongly affected by the C stratification processes.

Cover crop adoption, on the contrary, showed promise in the long term, whereas short-term outcomes (two-year experiment) were negatively affected by poor cover crop growth.

At the policymaker level, selection of sustainable land management practices will require multi-criteria tools to weigh the different management alternatives and find a compromise suitable to the needs and requirements of stakeholders. In terms of research, result differences between short (experimental data) and long timeframes (simulated data) indicate experimental studies are needed to calibrate models and evaluate SLM practices over the long term.

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# Chapter III - Have we reached the turning point? Looking for evidence of SOC increase under conservation agriculture and cover crop practices\*

\* **Camarotto C.**, Piccoli, I., Dal Ferro, N., Polese, R., Chiarini, F., Furlan L., and Morari, F. (2019). *Have we reached the turning point? Looking for evidence of SOC increase under conservation agriculture and cover crop practices*. European Journal of Soil Science (accepted for publication).

## 1. Introduction

Climate change mitigation through soil organic carbon (SOC) sequestration is largely related to agricultural lands (Lal, 2018). Global soils have been estimated to have lost a carbon (C) share of  $78 \pm 12$  Pg CO<sub>2</sub> since 1850, which can be compared to the  $270 \pm 30$  Pg CO<sub>2</sub> emitted by fossil fuel combustion during the same time (Aguilera *et al.*, 2013). Therefore, soil offers an enormous potential to recover the C that has been lost historically. For instance, the recent “4 per thousand” initiative aspires to reverse actual global SOC loss trends in agricultural soils towards increasing strategies at rates of 0.4% per year per 20 years (Chambers *et al.*, 2016), corresponding to approximately  $2.5$  Pg C y<sup>-1</sup> (Minasny *et al.*, 2017).

Among agricultural practices, those with high organic inputs, permanent soil cover and reduced or no-tillage are best suited to increase SOC stock and thus mitigate climate change impacts (Freibauer *et al.*, 2004; Autret *et al.*, 2016; Chenu *et al.*, 2018; Schwilch *et al.*, 2018). Conservation agriculture (CA) is based on three main pillars: minimum mechanical soil disturbance, maintenance of permanent soil covering and crop diversification (Hobbs *et al.*, 2008; Pittelkow *et al.*, 2015). CA increased in importance over time as a strategy to reduce SOC mineralization through minimum tillage or no-tillage and to increase C inputs with cover crops. The results from experiments comparing CA with conventional practices (CV) are still uncertain and depend on several factors: investigated soil depth, sampling methodologies, time interval, pedo-climatic variability, and crop type (Baker *et al.*, 2007; Xu *et al.*, 2016; Powlson *et al.*, 2016; Haddaway *et al.*, 2017; Steward *et al.*, 2018).

For example, West & Post (2002) estimated that a conversion to no-till system may enhance SOC sequestration by  $0.57 \pm 0.14$  Mg C ha<sup>-1</sup> y<sup>-1</sup> in the top 30 cm, with higher rates between five and ten years after adoption and a new equilibrium after 15 years. With deeper sampling, many studies have noted that the no-till system mostly affects the SOC vertical distribution within a profile rather than the total amount of SOC, with an increase in SOC in the top 10 cm and a decrease in SOC between 10 and 40 cm (Luo *et al.*, 2010; Ogle *et al.*, 2012). This observation was also confirmed by a recent meta-analysis conducted by Powlson *et al.* (Powlson *et al.*, 2014), which noted the potential impacts of no-tillage on SOC sequestration.

Interestingly, Chenu *et al.* (2018) highlighted that CA can enhance SOC stocks by increasing the C inputs rather than decreasing C mineralization. In addition, the authors identified major C accumulation with cover crops, especially when associated with no-tillage. The C input from either

crops or cover crops explained higher SOC stocks in the top 30 cm in CA when conducted for at least five years (Virto *et al.*, 2012). We obtained similar results in a 3-year transition period experiment aiming to compare CA and CV (Piccoli *et al.*, 2016) in silty-loam Cambisol and Fluvisol soils of the Veneto region in Italy. The SOC stock in the 0-30-cm layer was affected by root and residue input, although differences were not found when studying the soil down to 50 cm, most likely due to poor SOC physical protection mechanisms and thus poor SOC accumulation.

Studies do not all agree on the benefits provided by permanent soil cover with cover crops to increase SOC stocks. For instance, a recent meta-analysis by Poeplau and Don (2015) reported that 13 out of 139 analysed experiments showed an SOC stock decline after the introduction of cover crops. Authors speculated that the occurrence of a priming effect or inaccuracies in soil sampling surveys may affect SOC estimates when differences between treatments are small (Cambardella *et al.*, 1994).

The Veneto region, Northeast Italy, financed the application of both CA and cover crop practices with the purpose, among others, to increase SOC and reduce agricultural impacts from climate change.

In our previous experiment, the CA effect on SOC stocks was evaluated only over a 3-year transition period (Piccoli *et al.*, 2016). Moreover, although we were able to determine the effects of different C inputs on SOC stocks, the interaction of conventional tillage with cover crops (CC) was not tested.

After six years of adoption, we hypothesize that in comparison to CV, minimum mechanical soil disturbance, maintenance of permanent soil covering and crop diversification can enhance SOC stocks by offsetting the slow reaction capacity and poor SOC protection mechanisms of the Veneto plain soils. The present work aimed to assess the SOC stock variation due to the adoption of CA and CCs in comparison to CV within a large sample (i.e., 240) of 0-50-cm soil profiles.

## 2. Materials and methods

### 2.1. Experimental sites

The experiment was set up on three northeastern Italy farms (Figure 12). Farm 1 (F1), “Vallevecchia”, is located on the Adriatic coast ( $45^{\circ} 38.350' N$   $12^{\circ} 57.245' E$ , 2 m a.s.l.), and the soil is Gleyic Fluvisol or Endogleyic Fluvic Cambisol (FAO-UNESCO, 1990) with a texture ranging from silty-clay to sandy-loam. Farm 2 (F2), “Diana”, and farm 3 (F3), “Sasse Rami”, are located to the west, on the central ( $45^{\circ} 34.965' N$   $12^{\circ} 18.464' E$ , 6 m a.s.l.) and southern plains ( $45^{\circ} 2.908' N$   $11^{\circ} 52.872' E$ , 2 m a.s.l.), respectively. Both are characterized by Endogleyic Cambisol (FAO-UNESCO, 1990), silty-loam soil, which is more homogeneous in texture than the soil of F1 (Table 8).

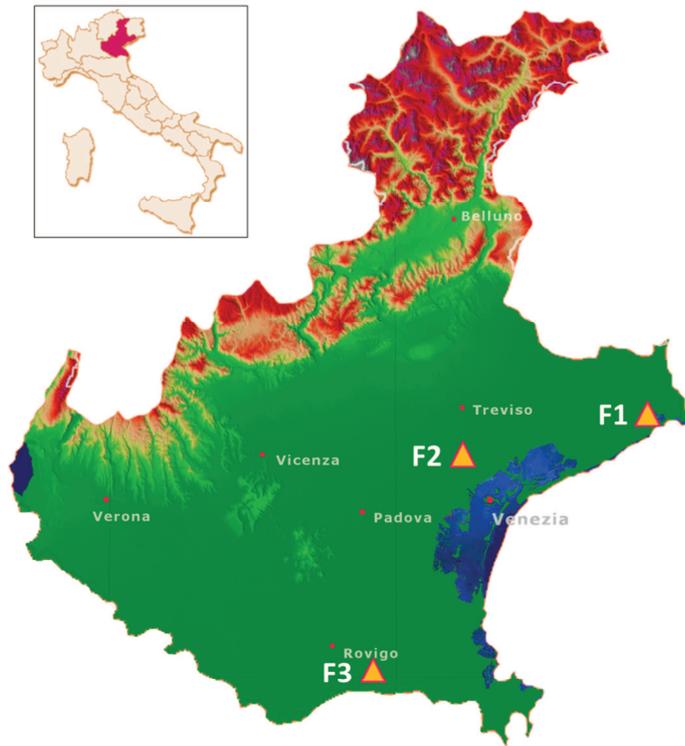


Figure 12 Experimental sites in the Veneto region low plain, northeastern Italy. Farm positions are marked with triangles. Farm 1 (F1), farm 2 (F2) and farm 3 (F3).

Property	Unit	Farm 1 “Vallevecchia”	Farm 2 “Diana”	Farm 3 “Sasse Rami”
Sand	g 100 g <sup>-1</sup>	34.2	8.3	18.4
Silt	g 100 g <sup>-1</sup>	42.6	66.1	57.8
Clay	g 100 g <sup>-1</sup>	23.2	25.6	23.8
pH		8.3	8	8.6
Carbonate	g 100 g <sup>-1</sup>	53	4	13
Active carbonate	g 100 g <sup>-1</sup>	3	1	3
Organic carbon	g 100 g <sup>-1</sup>	1	0.9	0.8
Assimilable P	mg kg <sup>-1</sup>	32	22	6
Exchangeable Ca	meq 100 g <sup>-1</sup>	24.7	21.7	15.5
Exchangeable Mg	meq 100 g <sup>-1</sup>	3.2	3.4	1.4
Exchangeable K	meq 100 g <sup>-1</sup>	0.5	0.3	0.2

Table 8 Main soil physical and chemical properties (0-50 cm) of the experimental farms.

The climate is sub-humid, with annual rainfalls of approximately 829 mm in F1, 846 mm in F2 and 673 mm in F3. In the median year, in F1, F2, and F3, rainfall was highest in autumn (302, 241 and 187 mm, respectively) and lowest in winter (190, 157 and 129 mm, respectively); temperatures increase from January (minimum average: -0.1, -0.9 and -0.2 °C, respectively) to July (maximum average: 29.6, 29.3 and 30.6 °C, respectively). In F1, F2, and F3, reference evapotranspiration (ET<sub>o</sub>) is 860, 816 and 848 mm, with a peak in July (4.9, 4.6 and 4.8 mm d<sup>-1</sup>). ET<sub>o</sub> exceeds rainfall from May to September in F1 and F2 and from May to October in F3.

## 2.2 Experimental design and treatments

The field experiment, established in October 2010 and still underway, compares CV with CA and CC practices. CC and CA systems were set up as the agri-environmental measure 214–sub-measure “i” (also called “Eco-compatible management of agricultural lands”) of the Veneto Region Rural Development Programme during the 2007-2013 period (Regione Veneto, 2013) based on the European Council Regulation (EC) No 1698/2005.

The same four-year crop rotation of winter wheat (*Triticum aestivum* L.) – oilseed rape (*Brassica napus* L.) – soybean (*Glycine max* (L.) Merr.) – maize (*Zea mays* L.) was initially used for all treatments. In 2015, the rotation was successively simplified to three years when oilseed rape cultivation was abandoned. In CA and CC, continuous soil cover was accomplished via cover crop

inter-cropping with sorghum-sudangrass (*Sorghum × drummondii* (Nees ex Steud.) Millsp. & Chase) in the spring-summer season and a vetch and barley mixture (*Vicia sativa* L. and *Hordeum vulgare* L.) in the autumn-winter season. This last crop has been replaced by winter wheat since 2015. Conversely, the soil remained bare between the main CV crops.

Rotation was in contemporary phases in the CA, CC and CV treatments. The experiment included a total of 44 fields: F1 and F2 had two fields under the CV treatment (8 in total instead of 4), each of which was close to the treatments with CA and CC. F3 had only one CV field. Experimental fields were rectangular (approximately 400 m length × 30 m width) with an average size of 1.2 ha.

Crop residues (in CC and CV) and cover crops (in CC only) acted as green manure and were incorporated into the soil with a 35-cm mouldboard plough, which was followed by 15-cm disc harrowing for seedbed preparation. The CA system was managed with no-tillage that left cover crop and crop residues on the soil surface.

The basal application of fertiliser was applied one to two weeks before sowing in CC and CV, whereas sub-surface band fertilisation was applied to CA during sowing. All systems were side-dressed with mineral fertilisers one time in maize and two times in wheat. No additional fertilisation was provided to the cover crops.

Pesticide applications, based on crop requirements, followed an integrated pest management programme and were the same for CV, CC, and CA. Prior to spring seeding, N-(phosphonomethyl) glycine was applied to suppress the winter cover crop in CA, while mechanical shredding was utilized to suppress the winter cover crop in CC. The summer cover crop was mechanically suppressed in both CC and CA treatments.

### **2.3 Crop residue and root biomass**

Crop and residue biomass were collected each year after harvesting for each treatment from three 2-m<sup>2</sup> and three 1-m<sup>2</sup> areas, respectively. The biomass samples were dried at 65 °C in a forced draft oven for 72 h for dry weight determination. The total root biomass in the upper 50-cm layer was determined according to the monolith method (Böhm, 1979) by excavating a 0.3 m × 0.3 m × 0.50 m soil cube in each sampling area from 2011 to 2015. In the following years (2016-2017), root biomass was estimated according to aboveground/belowground biomass ratios obtained from the previous years. Total C input was calculated as the sum of crop and cover crop residues and root inputs assuming a C concentration of 45% (Kätterer *et al.*, 2011).

## 2.4 Soil sampling and analysis

Two soil sampling campaigns were implemented, the first in spring 2011 and the second in spring 2017. Specifically, a hydraulic sampler was used to collect undisturbed soil cores (0-50 cm) from six systematically chosen locations in each field (Piccoli *et al.*, 2016). The same locations were identified across the years using a global navigation satellite system with real-time kinematic positioning (ca. 2-cm precision). The soil cores were cut into three distinct layers, 0-5 cm (L1), 5-30 cm (L2), and 30-50 cm (L3), and then stored at 5 °C until further physical and chemical analyses. A total of 1440 undisturbed soil samples were weighed, and a fraction (two-thirds) was oven-dried at 105 °C for 24 h for the bulk density (BD) calculation. The other soil fraction (one-third) was air-dried and sieved through 0.5-mm mesh to determine the organic C and total nitrogen (TN) content by flash combustion method using a CNS Elemental analyser (Vario Max, Analysensysteme GmbH, Langenselbold, DE) that followed an acid pre-treatment for inorganic C removal. The soil texture was determined with laser diffractometry (Malvern Mastersizer 2000, Malvern Instruments, Malvern, UK) of 2-mm sieved samples that were previously dispersed in a 2% sodium hexametaphosphate solution and shaken for 12 h at 80 rpm (Bittelli *et al.*, 2019).

The equivalent soil mass (ESM) method (VandenBygaart & Angers, 2006) was applied to normalize the effects of tillage on BD (Post *et al.*, 2001) for SOC and TN stock calculation. According to the minimum ESM method (Lee *et al.*, 2009), the equivalent SOC and TN stocks were calculated with the equation reported in Piccoli *et al.* (2016). The same equation was also used for TN stock. The minimum ESM was applied for incremental profiles, considering first L1 (0-5 cm, reference soil mass of 186 Mg ha<sup>-1</sup>), then L1 + L2 (0-30 cm, reference soil mass of 2384 Mg ha<sup>-1</sup>) and finally L1 + L2 + L3 (0-50 cm, reference soil mass of 5230 Mg ha<sup>-1</sup>). The stocks corresponding to the 5-30-cm and 30-50-cm soil layers were estimated by subtraction.

## 2.5 Statistical analysis

Data were analysed with a linear mixed-effect model based on the restricted maximum likelihood (REML) estimation method, considering clay and sand content as the continuous factors and treatment (i.e., CA vs CC vs CV), layer, year and farm (random factor) as categorical factors. Data from each treatment in the same field were considered sub-replicated and treated as nested measures. All possible first- and second-order interactions between factors were tested, and the model with the smallest Akaike's information criterion (AIC) was selected (Schabenberger &

Pierce, 2001). Post hoc pairwise comparisons of least-squares means (LSE) were performed using the Tukey method to adjust for multiple comparisons.

The treatment factor compared only the integrated effects CA vs CC vs CV; therefore, an additional mixed model was applied to determine the contributions of tillage and total C input on SOC stocks. The model considered C input (C from residues and root of main cover and cover crops) and sand and clay contents as continuous factors and tillage type (tillage vs no-tillage) as categorical factors.

Statistical analyses were performed with SAS software (SAS Institute Inc. Cary, NC, USA), 6.1 version.

### 3. Results

#### 3.1 Carbon input

Overall, cumulative C input (from both residues and root biomass of the main and cover crops) over the six-year monitoring period differed significantly among the treatments: CC had a higher value (24.0 Mg C ha<sup>-1</sup>) than those of CV and CA (19.4 Mg C ha<sup>-1</sup> on average) (Figure 13). Similarly, aboveground cumulative residue was significantly ranked as CC (16.3 Mg C ha<sup>-1</sup>) > CV and CA (12.7 Mg C ha<sup>-1</sup> on average). Sand negatively influenced residue biomass ( $p = 0.01$ ), while clay had no effects on it (Table 9). In contrast, the treatments did not discriminate root biomass, with slightly higher values in CC (7.7 Mg C ha<sup>-1</sup>) than in CA (6.8 Mg C ha<sup>-1</sup>) and CV (6.6 Mg C ha<sup>-1</sup>). Regarding root biomass, no specific interactions were observed with texture.

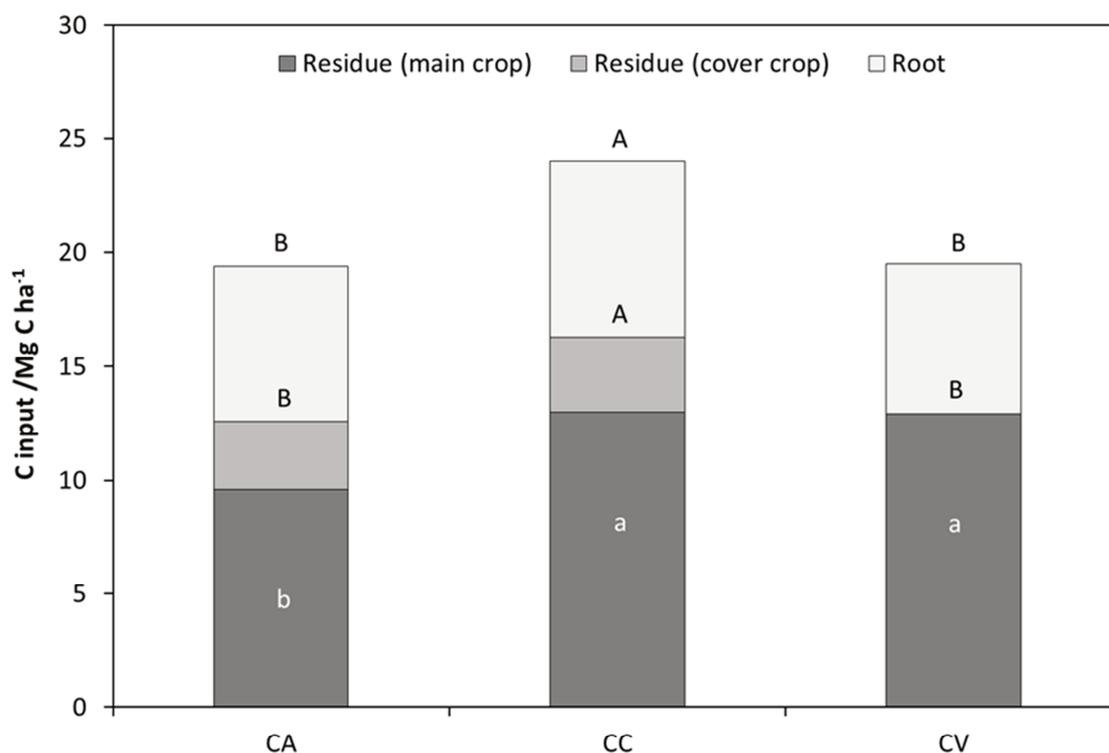


Figure 13 Cumulative C input (Mg C ha<sup>-1</sup>) from residues (main and cover crops) and roots. Values differ significantly when labelled with different letters (Tukey post hoc test with  $p \leq 0.05$ ). Uppercase letters indicate the significance of the cumulative value, and lowercase letters refer to the residue of the main crop. CA = conservation agriculture; CC = conventional tillage with cover crops; CV = conventional tillage.

Effect	Residue			Root	Total
	Main crop	Cover crop	Total		
Intercept	0.04	0.41	0.06	0.10	0.02
Treatment	<0.01	<0.01	0.01	0.31	0.02
Sand	0.01	0.38	0.01	0.59	0.02
Clay	0.52	0.76	0.62	0.59	0.66

Table 9 Comparison of significance levels among the linear mixed-effect model analyses of C input of residue and root biomasses.

### 3.2 Bulk density

BD showed significant differences ( $p < 0.01$ ) by depth, increasing from 1242 kg m<sup>-3</sup> at 0-5 cm to 1464 kg m<sup>-3</sup> at 5-30 cm and 1536 kg m<sup>-3</sup> at 30-50 cm, and the BDs of the treatments followed the order CA (1442 kg m<sup>-3</sup>) > CC (1403 kg m<sup>-3</sup>) > CV (1398 kg m<sup>-3</sup>). A denser layer was observed (“treatment × layer” interaction significant at  $p = 0.02$ ) in the CA subsoil (i.e., 5-30 cm), 1514 kg m<sup>-3</sup> (Figure 14), which was not found in the topsoil or in the deepest layer. Finally, clay content was negatively correlated with BD ( $p < 0.01$ ) (Table 10).

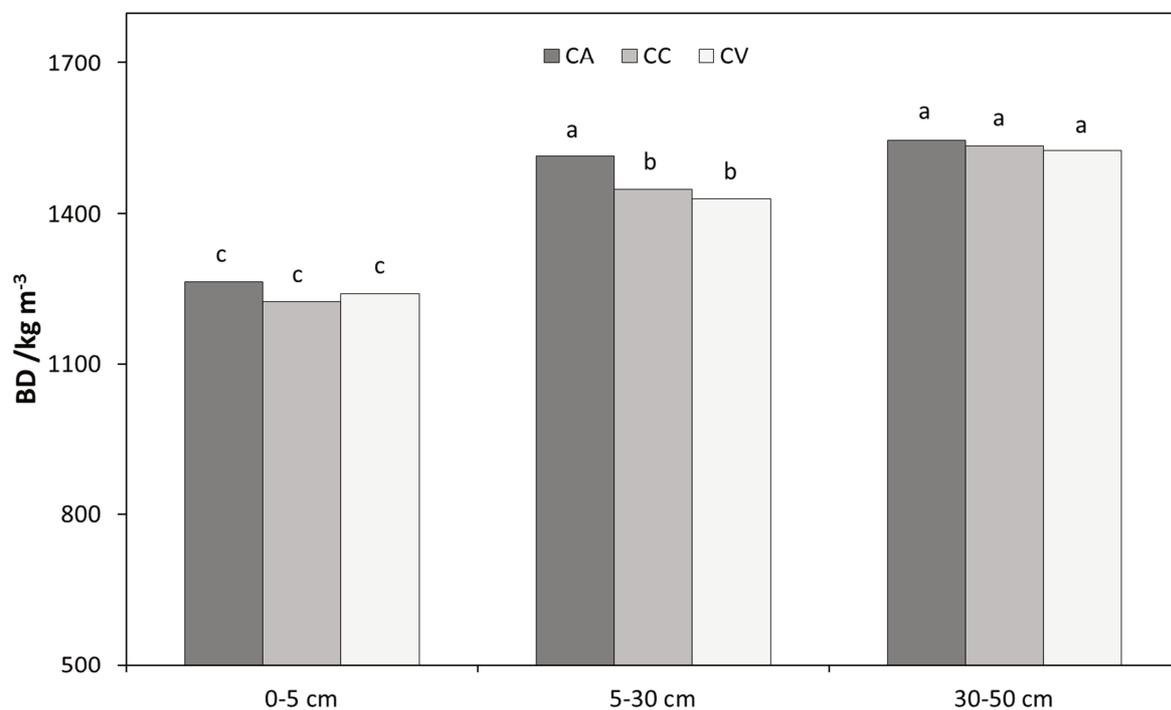


Figure 14 Soil bulk density (BD) at different soil layers (0–5 cm, 5–30 cm and 30–50 cm). Values differ significantly when labelled with different letters (Tukey post hoc test with  $p \leq 0.05$ ). CA = conservation agriculture; CC = conventional tillage with cover crops; CV = conventional tillage.

<b>Effect</b>	<b>Bulk density</b>	<b>SOC conc.</b>	<b>TN conc.</b>	<b>C/N</b>
Intercept	<0.01	0.02	0.03	0.01
Treatment	<0.01	<0.01	0.91	0.31
Year	<0.01	<0.01	0.02	<0.01
Layer	<0.01	<0.01	0.24	0.03
Sand	0.25	<0.01	<0.01	<0.01
Clay	<0.01	<0.01	0.83	0.01
Layer × Treatment	0.02	<0.01	-	-
Year × Treatment	0.06	<0.01	-	-
Year × Layer	0.27	<0.01	-	0.53
Year × Layer × Treatment	0.47	<0.01	-	-

- Variables not considered in the linear mixed-effect model according to lowest Akaike's information criterion.

Table 10 Comparison of significance levels among the linear mixed-effect model analyses of bulk density, soil organic carbon (SOC) concentration, total nitrogen (TN) concentration, and C/N ratio.

### 3.3 Soil organic carbon and total nitrogen concentrations

The SOC concentration showed significant differences ( $p < 0.01$ ) according to the “year × layer × treatment” interaction (Table 10). Low topsoil SOC values were observed in 2011, irrespective of the treatments ( $1.08 \text{ g C } 100 \text{ g}^{-1}$ , on average), while in 2017, CA yielded the highest SOC ( $1.51 \text{ g C } 100 \text{ g}^{-1}$ ), followed by CC ( $1.16 \text{ g C } 100 \text{ g}^{-1}$ ) and CV ( $1.02 \text{ g C } 100 \text{ g}^{-1}$ ) (Table 11). At deeper soil layers, no significant differences were observed among the treatments. SOC progressively decreased with depth, averaging  $0.99 \text{ g } 100 \text{ g}^{-1}$  at 5-30 cm and  $0.89 \text{ g C } 100 \text{ g}^{-1}$  at 30-50 cm (Table 11). The SOC concentration was also influenced by texture (Table 10) and was positively correlated with clay and negatively correlated with sand. Stronger relationships were found in F1 (Table 12), where irrespective of the year, the SOC concentration exhibited a correlation coefficient  $\geq 0.74$  with clay and  $\leq -0.78$  with sand ( $p \leq 0.01$ ). In contrast to F1, F2 and F3 showed weaker correlations with  $r$ , at  $< 0.56$  for clay and  $> -0.52$  for sand.

Treatment	Year	SOC /g 100 g <sup>-1</sup>					
		0-5 cm		5-30 cm		30-50 cm	
CA	2011	1.11	bc	0.98	cdefg	0.88	g
	2017	1.51	a	0.97	defg	0.90	fg
CC	2011	1.10	bc	1.04	bcd	0.91	efg
	2017	1.16	b	0.99	cdefg	0.87	g
CV	2011	1.02	cde	0.97	defg	0.89	g
	2017	1.02	cde	1.01	cdef	0.89	g

Table 11 Soil organic carbon (SOC) concentration at different soil layers (0–5 cm; 5–30 cm; 30–50 cm) in 2011 and 2017. Values differ significantly when labelled with different letters (Tukey post hoc test with  $p \leq 0.05$ ). CA = conservation agriculture; CC = conventional tillage with cover crops; CV = conventional tillage.

Variable	Farm	Year	Clay	Sand
SOC	F1	2011	<b>0.77</b>	<b>-0.80</b>
		2017	<b>0.74</b>	<b>-0.78</b>
	F2	2011	<b>0.25</b>	-0.07
		2017	<b>0.20</b>	<b>-0.16</b>
	F3	2011	<b>0.56</b>	<b>-0.52</b>
		2017	<b>0.32</b>	<b>-0.34</b>

Table 12 Correlation coefficients ( $r$ ) between soil organic carbon (SOC) concentrations and texture (sand and clay content) at the three farms (F1, F2 and F3) and in the two years of sampling (2011 and 2017). Values in bold are significant at  $p \leq 0.01$ .

The TN concentration did not discriminate between treatments and depths (Table 10), whereas differences were observed with the year ( $p = 0.02$ ), increasing from 0.158 g N 100 g<sup>-1</sup> in 2011 to 0.180 g N 100 g<sup>-1</sup> in 2017. Although not significant, a higher TN increase was found in CA (+14.1%) and CV (+10.0%) than in CC (+3.1%) and in the top layer (+16.9%) compared to in the deeper layers (+5.0% at 5-30 cm and +4.1% at 30-50 cm). Sand also negatively affected soil TN ( $p < 0.01$ ).

In addition, the C/N ratio did not discriminate between treatments (Table 10), with an average value of 6.4. In contrast, the C/N ratio varied with depth ( $p = 0.03$ ) and was higher in the topsoil 0-5 cm (C/N = 6.5) than in the 30-50 cm layer (C/N = 6.2), and the C/N ratio varied with year ( $p < 0.01$ ),

decreasing from 6.5 in 2011 to 6.2 in 2017. Although not significant, a higher C/N decrease was found in CV (-7.7%) than in CA and CC (-3.4% and -3.0%, respectively) and in the top layer (-7.1%) than in the deeper layers (-3.1% at 5-30 cm and -3.9% at 30-50 cm).

### 3.4 Soil organic carbon and total nitrogen stock variations

The SOC stocks and their variations between 2017 and 2011 were tested at 0-5-cm, 0-30-cm, and 0-50-cm soil profiles using the ESM method.

In 2017, SOC stocks were 2.72 Mg C ha<sup>-1</sup>, 2.20 Mg C ha<sup>-1</sup> and 1.81 Mg C ha<sup>-1</sup> at 0-5 cm; 25.57 Mg C ha<sup>-1</sup>, 25.48 Mg C ha<sup>-1</sup> and 23.17 Mg C ha<sup>-1</sup> at 0-30 cm; and 53.64 Mg C ha<sup>-1</sup>, 51.73 Mg C ha<sup>-1</sup> and 49.81 Mg C ha<sup>-1</sup> at 0-50 m in CA, CC and CV, respectively.

Differences in SOC stock variations were found in the top layer (0-5 cm), where CA significantly increased ( $p < 0.01$ ) the SOC stock by 0.74 Mg C ha<sup>-1</sup> in comparison to the SOC stocks of CC (0.08 Mg C ha<sup>-1</sup>) and CV (-0.01 Mg C ha<sup>-1</sup>) (Figure 15). CA increased the SOC stock over time even at the 0-30 cm (2.34 Mg C ha<sup>-1</sup>) and 0-50 cm profiles (2.49 Mg C ha<sup>-1</sup>). In contrast, a depletion of -0.86 Mg C ha<sup>-1</sup> and -2.44 Mg C ha<sup>-1</sup> was observed in CC, corresponding to -0.14 Mg C ha<sup>-1</sup> and -0.41 Mg C ha<sup>-1</sup> y<sup>-1</sup>, respectively. In contrast, differences between CA and CV decreased progressively at increasing depths, with 65% ( $p = 0.09$ ) at 0-30 cm and 20% ( $p = 0.94$ ) at 0-50 cm (Figure 15).

Individual effects of management practices on SOC stock variations were evaluated by applying a linear mixed-effect model that included total C input and sand and clay contents as the continuous factors, and tillage as the categorical factor (Table 13). The results revealed that topsoil SOC stock variation was affected by both tillage ( $p < 0.01$ ) and C input ( $p = 0.05$ ), whereas texture had no effect. Considering deeper soil profiles, C inputs were not significant at depths of 0-30 cm and 0-50 cm, while tillage significantly affected the 0-30 cm soil layer with higher stock variation with no-tillage (2.20 Mg C ha<sup>-1</sup>) than with tillage (0.21 Mg C ha<sup>-1</sup>) (Table 13). By considering the distinct layers, tillage was still significant at the 5-30 cm layer, while in the deepest layer (30-50 cm), only C input negatively affected ( $p = 0.04$ ) the SOC stock variation.

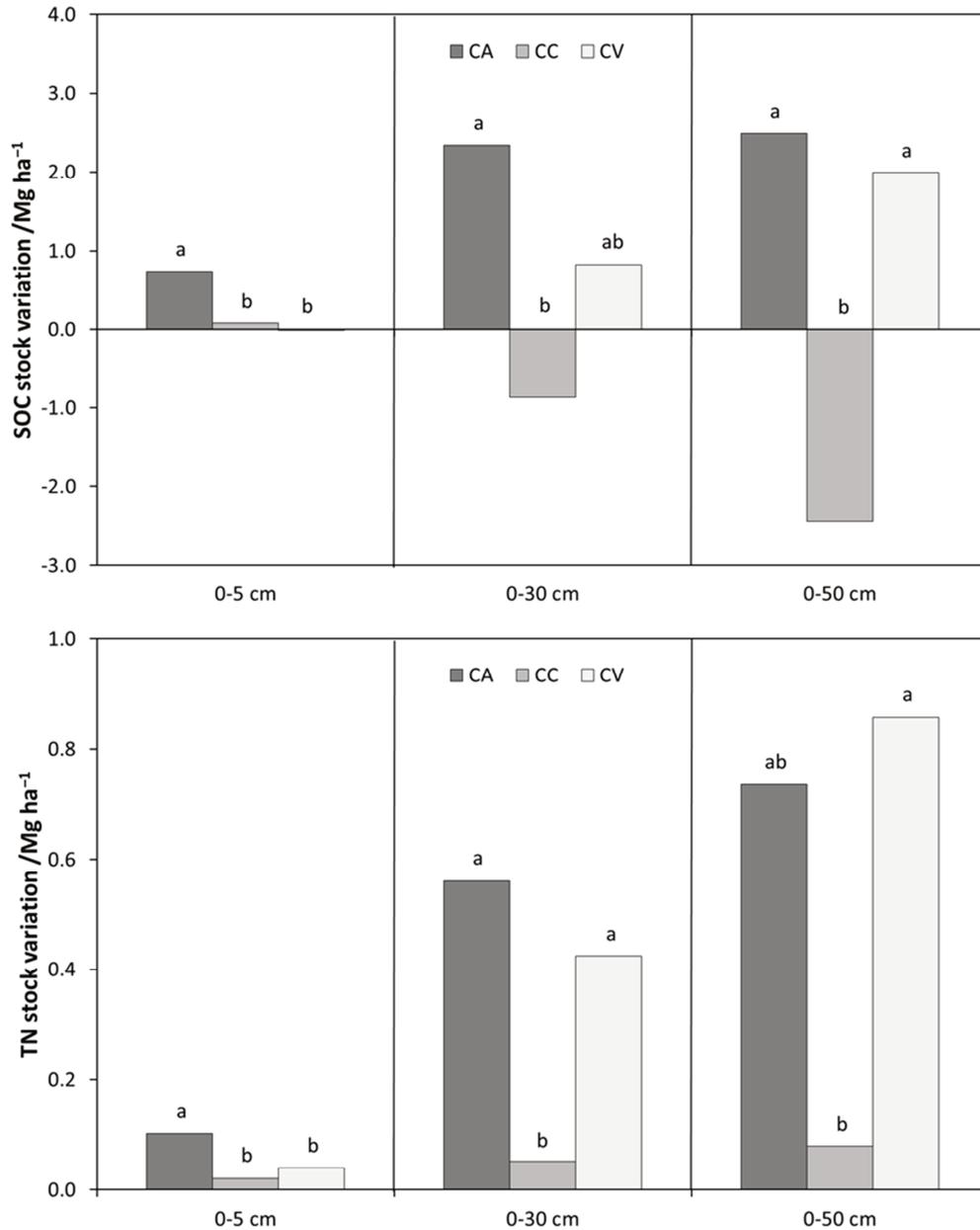


Figure 15 Soil organic carbon (SOC) (top) and total nitrogen (TN) (bottom) stock variations from 2011 to 2017 at different soil profiles (0–5 cm, 0–30 cm and 0–50 cm). Values differ significantly when labelled with different letters (Tukey post hoc test with  $p \leq 0.05$ ). CA = conservation agriculture; CC = conventional tillage with cover crops; CV = conventional tillage.

TN stock variations were affected by the treatments in all cumulative soil profiles (Figure 15). Similar stock dynamics to those observed for SOC were found in CA and CV, which accumulated TN over the six-year period. In contrast, a different pattern was observed for the CC treatment that showed a slight TN accumulation with respect to previously reported SOC depletion. TN stock showed higher ( $p < 0.01$ ) topsoil variation in CA ( $0.10 \text{ Mg N ha}^{-1}$ ) than in both CC and CV

(0.03 Mg N ha<sup>-1</sup>, on average) and was present in significantly lower values in CC in the intermediate layers (0-30 cm) than in other layers. In the 0-50 cm layers, significant differences were only observed between CC and CV (0.08 Mg N ha<sup>-1</sup> vs 0.86 Mg N ha<sup>-1</sup>), while CA had intermediate values (Figure 15).

<b>Effect</b>	<b>SOC stock variation</b>				
	<b>0–5 cm</b>	<b>5–30 cm</b>	<b>30–50 cm</b>	<b>0–30 cm</b>	<b>0–50 cm</b>
Intercept	0.57	0.75	0.22	0.72	0.34
Tillage	<0.01	0.06	0.46	0.01	0.41
C input	0.05	0.82	0.04	0.97	0.21
Sand	0.08	0.46	0.02	0.50	0.10
Clay	0.42	0.73	0.73	0.80	0.94

Table 13 Comparison of significance levels among the linear mixed-effect model analyses of soil organic carbon (SOC) stock variation in the 0–5 cm, 5–30 cm, 30–50 cm, 0–30 cm and 0–50 cm soil layers.

## 4. Discussion

Agricultural management strategies to accumulate SOC stocks can act by both increasing C inputs and enhancing SOC protection mechanisms that reduce C mineralization.

With regard to C addition, management of arable land with soil cover (i.e., CC) significantly increased C inputs with respect to CA and CV, estimated at +23%. CV and CA had similar C inputs, although the latter also included cover crop-derived C. These results were primarily due to differences in the main crop residues that were lower in CA than in CC and CV. Thus, the adoption of no-tillage was the primary factor that reduced the main crop biomass production, probably due to poor seed germination (Constantin *et al.*, 2010). Cover crops were not able to offset that negative effect by providing a significant amount of aboveground C input. The C input from cover crops varied considerably from one year to another due to unpredictable weather conditions and narrow cultivation windows. Similar findings were previously found in an experiment in F3 by Camarotto *et al.*, (2018). Moreover, some negative effects on soil functions and crop growth were likely due to the increased soil BD, especially in soils with poor structure stability and low SOC (Piccoli *et al.*, 2017).

These findings were in agreement with those of Piccoli *et al.* (2016), which observed higher BD in the top 30 cm of CA over a three-year period in the same experimental sites presented in this study. We observed similar results in the 5-30-cm layer where BD increased under CA, while topsoil (i.e., the 0-5-cm layer) was not affected, probably due to topsoil accumulation of crop residue and shallow root growth (Kay & VandenBygaart, 2002). This result seems to confirm the findings of Dolan *et al.* (2006) and Vogeler *et al.* (2009), which highlighted BD levelling over time in topsoil between no-tillage and tillage systems.

Differences in SOC concentrations between treatments were found only in the 0-5-cm layer, with higher values in CA than in CC and CV. Some authors (Ogle *et al.*, 2012; Powlson *et al.*, 2014) have argued that the retention of crop residues in the field and its association with no-tillage practices may impact SOC content and dynamics in several ways: i) reduce SOC mineralization rate due to low residue-SOC mixing and accentuated mulching, which reduce exposure to soil microbial attacks, decrease soil surface temperature and aeration and increase aggregate stability (Duiker & Lal, 2000; Bronick & Lal, 2005; Al-Kaisi *et al.*, 2005); ii) decrease subsoil C movement due to limiting burial of residues and SOC-rich topsoil layers; and iii) increase SOC stratification as a result of a change in soil physical properties that hinder root deepening and promote surface

accumulation (Qin *et al.*, 2004; Martínez *et al.*, 2008). In this context, Dwyer *et al.* (1996) found that root biomass was not significantly different between CV and no-tillage systems – as also observed in our study – despite being strongly stratified under no-tillage management.

CC showed different SOC concentration dynamics than those observed in CA because only a slight increase was found in the topsoil and a decrease was found in the subsoil. Although numerous studies have shown that cover crops can increase long-term SOC concentrations (Lal, 2004; Poeplau & Don, 2015b), the effects of cover crops may not be detectable in the first years after their establishment (Acuña & Villamil, 2014; Blanco-Canqui *et al.*, 2014).

The positive correlation found between SOC and clay content confirmed the importance of physical protection mechanisms on SOC dynamics (Six *et al.*, 2002; Xu *et al.*, 2016), regardless of treatment. In particular, this scenario was emphasized in F1, despite being characterized by lower clay than that in F2 and F3. Interestingly, Minick *et al.* (2017) suggested that the stabilization of SOC is influenced by clay mineralogical composition, especially in calcareous soils. Indeed,  $\text{Ca}^{2+}$  interacts with SOC through inner- and outer-sphere bridging processes and protects SOC through aggregate stabilization and sorption mechanisms. In fact, in this study, the SOC- $\text{Ca}^{2+}$  relationship was detected only in farm F1. As previously reported by Piccoli *et al.* (2016), in this location, the soil mineralogical composition is characterized by high levels of carbonates in the clay fraction, in particular calcite and dolomite, which may confirm  $\text{Ca}^{2+}$ -mediated SOC stabilization (Rowley *et al.*, 2018).

SOC stock variation within the soil profile differed between treatments. Significant differences between CA and CV were observed only at the 0-30 cm layer, emphasizing that a different SOC vertical distribution, rather than a substantial accumulation, occurred over the 6-yr period (Baker *et al.*, 2007; Luo *et al.*, 2010; Ogle *et al.*, 2012; Palm *et al.*, 2014; Powlson *et al.*, 2016). The results confirmed the previous three-year findings of Piccoli *et al.* (2016), which showed significant differences between the CA and CV treatments in the 0-30 cm soil profile but not in the 0-50 cm soil layer. The average rate of SOC accumulation at 0-30 cm was  $0.24 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$ , a value similar to the  $0.25 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$  observed in our experiment. The results suggest that a six-year transition period was not sufficient to increase the SOC stock with respect to CV in the whole 0-50 cm profile, a result also observed by Govaerts *et al.* (2009). However, some different SOC stock dynamics were observed over time, although the results were not significant; the stock variations were higher in CV than in CA in 2014 (Piccoli *et al.*, 2016), the reverse in 2017.

Despite the higher organic C addition in CC, as provided by C input with both crops and cover crops, SOC stock was depleted over time compared to small SOC stock increases in CA and CV. Organic C stock variations substantially changed with depth, estimated at -143% in the 0-30 cm layer and -203% in the 0-50 cm layer in CC, indicating that some SOC bioturbation mechanisms may occur. According to several authors (Fontaine *et al.*, 2007; Chenu *et al.*, 2018), a priming effect is likely when fresh biomass-C with high decomposition rates (Kätterer *et al.*, 2011) enters a soil system, inducing a negative C balance. Therefore, the supply of fresh plant material from cover crops would likely have accelerated the SOC mineralization dynamics of slow-cycling ancient C, especially in the deep horizons where microorganisms activity is limited by energy. Blagodatskaya & Kuzyakov (2008) found that the priming effect depends on both the biomass input and soil microbial amounts, highlighting major SOC depletion when the input/microbial C ratio is above 0.50. In contrast, higher C additions than microbial biomass C (ratio > 2) would not lead to microbial SOC utilization. Here, by estimating a microbial biomass of 170 mg kg<sup>-1</sup> after three years (Piccoli *et al.*, 2016) and average fresh biomass-C addition of 0.57 Mg ha<sup>-1</sup> yr<sup>-1</sup> with cover crops, we similarly found an input/microbial C ratio of 0.46 that would have stimulated the growth of microorganisms and real SOC mineralization. Moreover, SOC depletion would be emphasized with C-rich biomass inputs associated with low N availability (both in soil and added biomass): microorganisms would have acquired nitrogen by decomposing soil organic matter, in turn producing a priming effect (Blagodatskaya & Kuzyakov, 2008). Indeed, the only CC treatment buried fresh biomass-C with a high C/N ratio, especially sorghum-sudangrass, that represented 70% of total fresh biomass input. This phenomenon is supported by observations of nitrogen stock variations that remained essentially stable in CC over six years, whereas they increased in both CA and CV. Instead, the soil C/N ratio did not distinguish microbial-mediated C dynamics among treatments, because differences in microbial diversity did not alter the rate of C and N mineralization (Nannipieri *et al.*, 2003). The above scenario is partially confirmed by the results of the linear mixed-model analyses that revealed that in the deepest layer (30-50 cm), where plant residues were buried, C input had a significant and negative impact on SOC stock.

In contrast, the priming effect was not detected in CA, where the main deep C input was provided by roots, whose humification coefficient can be expected to be approximately 1.9 times higher than that of aboveground plant materials, as estimated in similar pedo-climatic conditions (Berti *et al.*, 2016). A priming effect was also not observed in CV whose buried biomass – from the main crop only – contained dried material less reactive to microbial attacks (Kumar & Goh, 1999).

Nevertheless, the relationship between C inputs and tillage practices is far from completely understood. Linear mixed-model analyses revealed that no-tillage influenced SOC accumulation within the 0-30 cm soil profile, partially contradicting the previous findings of Chenu *et al.* (2018), which identified a pivotal role of C input through cover crops under conservation management practices. Many studies have shown a correlation between C input and long-term SOC stock variations (Halvorson *et al.*, 2002; Barbera *et al.*, 2010), although the SOC storage is determined by not only the return of C to soil but also the balance between input and output (Sainju *et al.*, 2002). Ogle *et al.* (2012) highlighted that the transition to a no-tillage system can increase the mean residence time of SOC by 15% and therefore assumed that an input of C could decrease by 15% before causing a reduction in SOC stocks. Consequently, the ability of conservation practices to preserve – or increase – SOC is also driven by a reduction in SOC mineralization rates (Al-Kaisi *et al.*, 2005). This scenario was partially confirmed by the combined effect of no tillage and C input on SOC stock variations, integrating previous results obtained by Piccoli *et al.* (2016) in the 3-yr transition period, which showed that C input was the main factor affecting SOC in the first 30 cm.

## 5. Conclusions

The 6-year time span considered here for comparing CA and CC as mechanisms to increase SOC stocks with respect to the SOC stocks of CV revealed that a turning point was not reached; thus, the starting hypothesis was rejected. Expansive field surveys and deep soil sampling to a depth of 50 cm showed that CA enhances SOC stratification rather than SOC accumulation, with high topsoil SOC that may have partly counteracted soil surface compaction. However, a comparison with previous SOC stock quantifications between CA and CV after three years of the experiment suggests that some SOC stock increase occurred, even at 50 cm, despite being not significant. The burial of fresh biomass-C with cover crops in arable systems (CC) enhanced SOC stock depletion most likely due to priming effects, suggesting that C input management is pivotal for its accumulation in agroecosystems with low soil fertility and low SOC protection capacity. If these results will be confirmed by further experiments, higher cover crop biomass production than observed experimentally as well as the use of legume cover crops will be required, which would limit the microbial-driven SOC mineralization and soil N depletion. However, this research, and its comparison with results from previous studies, does not conclusively provide an understanding of SOC stock dynamics, which suggests the need for longer monitoring studies.

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## **Chapter IV – General conclusions**

In this thesis we aimed to evaluate two sustainable land management practices, such as conservation agriculture (CA) and cover cropping (CC), in relation to the ecosystem services offered in the low-lying venetian plain.

The results of field experiments for both practices were contrasted, varying according to soil functions, ecosystem service category and evaluation time span considered.

Conservation agriculture showed to be more effective than conventional agriculture in providing short-term regulating ecosystem services regarding the improvement of water quality, reducing percolation and leaching of nutrients to groundwater, and air quality, with lower greenhouse gas emissions, but less effective in the long term, at least for greenhouse gas mitigation. With regard to an increase in stocks of soil organic carbon, the six-year period considered in this study was not sufficient to reveal a real benefit from conservation agriculture, showing that it tends to stratify the SOC rather than increase its accumulation. In this context, a turning point - i.e. the change from SOC stratification to accumulation – was not reached. However, a comparison with previous SOC stock quantifications between CA and CV after three years of the experiment suggests that some SOC stock increase occurred, despite being not significant. In contrast, conservation agriculture has led to a significant reduction in the main crop yields.

The adoption of cover crops showed promising results in the long term, with lower greenhouse gas emissions compared to conventional agriculture, while the short-term results did not show substantial differences with the control with regard to the improvement of water and air quality, while they showed worse results on carbon dynamics, with a sharp decrease in the SOC content six years after the establishment of the management. The burial of fresh biomass-C most likely enhanced SOC stock depletion most likely due to priming effects, suggesting that C input management is pivotal for its accumulation in agroecosystems with low soil fertility and low SOC protection capacity.

This holistic evaluation of conservation agriculture and cover crop practices in Veneto Region confirmed, at the moment, the lack of a “perfect” solution that was able to deliver both provisioning (e.g. food production) and regulating services (e.g. water and air quality). Furthermore, a number of ecosystem services delivered in the short and medium term might even be reversed over the long term, and *vice versa*. The field experiments clearly indicated the need for a longer transition period to reach a favourable equilibrium in the CA and CC systems of the low-lying venetian plain in order to exploit the benefits provided by such managements. Further studies are therefore needed to disentangle the factors that govern long- and short-term soil-related dynamics, in order to identify

any transitional effects produced by these management practices. Moreover, future research should include the assessment of a wider range of ecosystem services (e.g. lifecycle maintenance, habitat and gene pool protection) as well as all the stages of agroecosystem management for an overall assessment of SLM benefits and drawbacks (e.g., through Life Cycle Assessment).

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