1	Agro-environmental aspects of conservation agriculture compared to conventional systems: a
2	3-year experience on 20 farms in the Po valley (Northern Italy)
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23	Keywords: conservation agriculture; soil organic carbon; soil fertility; economic profitability; cover
24	crop

#### 25 Abstract

An evaluation of the effect of the conservation agriculture (CA) on agro-environmental aspects is 26 needed at the farm scale in intensive production systems, which are likely prone to reduce soil 27 fertility. Here, as part of the HelpSoil LIFE+ Project and involving 20 farms in the Po valley 28 (Northern Italy), we have estimated the soil organic carbon (SOC) content, SOC stock, crop yield, 29 biological fertility, soil biodiversity, and economic efficiency under different agricultural systems 30 (CA and conventional, CvtA) at the beginning (March 2014) and end (October 2016) of the 31 experimental period. CA was mostly represented by no-till practice (NT) coupled with the 32 cultivation of winter cover crops. Minimum tillage (MT) was considered as CA or CvtA practice 33 according to the farm design. The CA practices have been implemented on the monitored farms at 34 35 different times (Long-term=before 2006, Medium-term=between 2006 and 2013, Short-term=after 36 2013). A direct comparison between CA and CvtA of soil-related variables, yields, and costs was performed on 14 out of the 20 farms; data were statistically treated with a linear mixed model. 37 Overall, CA resulted in significantly higher SOC content, SOC stock, biological fertility, QBS-ar, 38 and earthworms for the Medium-term group. Considering the effect of tillage practices observed on 39 the 20 farms, SOC content was the highest in NT for the Long-term group. The biological fertility 40 index was higher in NT and MT compared to CvtA within the Long-term and Medium-term groups 41 in 2016. QBS-ar was the higher in MT and NT than CvtA for the Long-term and Medium-Term 42 groups. The number of earthworms was the highest under NT for the Long-term group. Maize, 43 winter wheat, and soybeans yields were generally 1 t ha<sup>-1</sup> higher in CvtA than in CA, but this did 44 not reach statistical significance. The cost for herbicides was 18% more expensive in NT, whereas 45 the fuel consumption and total costs for weeding operations did not differ between NT and CvtA. 46 The overall outcome of the analysis was that CA is a viable solution for intensive farms in the 47 monitored area, but further skills need still to be acquired in to enhance its economic feasibility. 48

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## 50 Highlights

- Conservation and conventional agriculture (CA, CvtA) evaluation on 20 farms in 3 years
- 52 Implementation\_Year (IY) of CA differed among the farms
- SOC and biological fertility index were higher in CA than in CvtA according to IY
- CA of recent IY was found in higher SOC storage and lower yield than CvtA
- CA may be more profitable than CvtA if cover crop and weed management improves

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## 57 **1. Introduction**

Agriculture is required to face major agro-environmental threats, such as the increasing carbon 58 59 dioxide (CO<sub>2</sub>) concentration in the atmosphere, decreasing biodiversity, and limited water availability (Bouma and McBratney, 2013); these processes can be effectively mitigated by an 60 adequate management of the soil (McBratney et al., 2014). Conservation agriculture (CA), 61 originally aimed at reducing water and wind erosion in the United States (Faulkner, 1943), was 62 recently promoted by the Common Agricultural Policy of the European Union (CAP, Rural 63 64 Development Programme 2014 -2020; Basch et al., 2011) to tackle the above mentioned agroenvironmental issues (Armengot et al., 2016). CA consists in a set of agronomic practices, which 65 includes: (1) minimal soil disturbance, performed through several techniques, i.e. minimum tillage 66 67 (MT) and no-till (NT); (2) permanent soil cover that is ensured by retaining crop residues and (3) adopting crop rotations that include cover crops (Palm et al., 2014). Integrated nutrient management 68 was indicated by Lal (2015) as another conceptual principle of the CA systems, obtained by nutrient 69 70 recycling, biological nitrogen fixation, and cautious use of chemical fertilizers, contributing to a 71 sustainable production system.

Diverse reviews dealing with the evaluation of the benefits determined by the switch from
conventional agriculture (CvtA) to CA in temperate regions have been published in the last decade;

contrasting results are shown in these reviews, suggesting that such a conversion does not always 74 75 resulte in environmental and economic benefit (Table 1). The data reported by Virto et al. (2012), Abdalla et al. (2013), and Stavi et al. (2016) show that the contribution of CA practices, namely NT, 76 77 on carbon sequestration is higher than under CvtA. Conversely, Ogle et al. (2012) stated that SOC sequestration is even lower under reduced tillage than in CvtA, while other authors reported no 78 difference between the two systems (Aguilera et al., 2013; Palm et al., 2014; Ranaivoson et al., 79 80 2017). According to Powlson et al. (2014), the apparent increase in soil organic carbon (SOC) under NT when compared to CvtA is mostly due to the redistribution of C in the topsoil and does not 81 necessarily lead to a net increase in SOC stock. Moreover, time after CA implementation is a factor 82 83 affecting SOC increase (Pittelkow et al., 2015). Evidences from a study conducted by Virto et al. (2012) suggest that the variability in SOC storage induced by the conversion to NT, seems mostly 84 due to the crop production variability as crop C input differences was the only factor significantly 85 86 explaining (about 30% of the total variability) the SOC stock increase in NT compared to CvtA. Ogle et al. (2012) analyzed 74 published studies carried out in the United States and found that the 87 88 adoption of NT in conventionally managed croplands might reduce SOC stocks. On the contrary the role of cover crops combined with NT in increasing SOC storage has been reported by many 89 authors (Aguilera et al., 2013; Palm et al., 2014; Lal et al., 2015). 90

91 Evidence of a reduction of greenhouse gases emissions by CA has been reported by many authors (Morris et al., 2010; Corsi et al., 2012; Perego et al., 2016). Concerning the nitrous oxide, (N<sub>2</sub>O) 92 which is acknowledged as one of most potent gas in terms of warming potential, it was highlighted 93 that CA has a similar or negative effect on N<sub>2</sub>O emissions relative to CvtA (Abdalla et al., 2013; 94 95 Palm et al., 2013; Powlson et al., 2014). Available data suggest that the switch from CA to CvtA practices is likely to enhance soil biodiversity (Knowler and Bradshaw, 2007; Powlson et al., 2014). 96 97 In particular, Van Capelle et al., (2012), in a review focused on German data, found that, although microorganisms generally benefit from a reduction of tillage intensity, tillage effects on soil 98

99 organisms vary according to soil texture, and therefore tillage system should be chosen on the basis100 of the local soil characteristics.

Positive effects on soil structure were reported by several authors in recent reviews (Hobbs et al., 101 2008; Morris et al., 2010; Abdalla et al., 2013; Powlson et al., 2014). It was acknowledged that 102 progressive formation of macropores might compensate the soil compaction occurring in the first 103 phase of NT implementation due to roots and faunal activity with time (Kay and Vann, 2016). 104 105 Moreover, the residue retention on the soil surface creates optimal conditions for macrofauna (Mutema et al., 2013) and particularly for earthworms (Briones and Schmidt, 2017), which in turn 106 promote the development of soil structure. Moreover, soil structural and hydraulic properties benefit 107 108 from the cover crops cultivation (Blanco-Canqui et al., 2015).

Various aspects concerning farm management are regarded as critical in the adoption of the CA 109 practices; both positive and negative effects have been listed in reviews published in the last decade 110 111 (Table 1). Hobbs et al. (2008) and Scopel et al. (2013) stated that farm labor is saved by reducing tillage operations and the associated fuel consumption. Conversely, Powlson et al. (2014) reported 112 that suitable machinery for planting under conservation tillage might not be available, especially for 113 small farmers in less developed countries; in addition extra labor or use of herbicides for weed 114 control might imply increasing costs. Many authors acknowledged the negative effect of CA on 115 116 weed control in other reviews (Knowler and Bradshaw, 2007; Stavi et al. 2016; Ranaivoson et al., 2017). In the calculation of the economic balance, a critical variable is the revenue generated by 117 yield, which is generally lower under CA than CvtA (Ogle et al., 2012; Powlson et al., 2014; 118 Pittelkow et al., 2015), but might be similar (Knowler and Bradshaw, 2007; Hobbs et al., 2008; 119 Stavi et al. 2016; Ranaivoson et al., 2017) or slightly higher (Farooq et al., 2011) due to the crop 120 121 residues retention. Blanco-Canqui et al. (2015) reported that cover crop cultivation under CA often results in yields that do not differ from those under CvtA. 122

123 The overall outcome of the examined reviews and papers is that it is required to define farm-124 specific options to boost the potential of CA and to reduce environmental and economic drawbacks.

Recently, multiple studies have addressed CA in tropical, sub-tropical, and arid regions, with a 125 126 focus on smallholder farmers (Giller et al., 2015; Brown et al. 2107a, Brown et al., 2017b, Farris et al., 2017). However, the implementation of CA in Europe (Soane et al., 2012), and in particular in 127 Italy, is still an ongoing process whose environmental and economic feasibility needs to be 128 evaluated. In Italy, CA is currently supported by the EU's CAP and the rural development 129 programmes of the Italian administrative regions, such as the five regions laying in the Po valley 130 131 (Piedmont, Lombardy, Emilia-Romagna) and Veneto and Friuli Venezia Giulia plain (Northern Italy). For simplicity, hereafter in the text, the entire flat area is referred to as Po valley. In this area, 132 the organic carbon stock stored in the topsoil (i.e. 0.3 m depth) is 34 to 60 Mg ha<sup>-1</sup> and the potential 133 for further uptake is estimated to be at least 12.8 Mg ha<sup>-1</sup> of CO<sub>2</sub> equivalent if soils are managed 134 appropriately (Brenna et al., 2014). The Europe Commission funded the HelpSoil project (LIFE12 135 ENV/IT/000578), which was carried out on 20 farms in the Po valley and aimed at: i) strengthening 136 the ecological functions of the soils (e.g. carbon sequestration, increase of fertility and edaphic 137 biodiversity, protection against erosion); ii) evaluating the opportunity of coupling subsurface drill 138 139 irrigation and no-till to enhance water use efficiency; iii) improving the nitrogen use efficiency 140 while testing various fertilization techniques, in particular in the usage of livestock slurry; and iv) reducing the use of pesticides for the control of plant pests and diseases. The present study was set 141 142 within the framework of the HelpSoil Project, under the assumption that only the on-site assessment of environmental and economic variables enables a sustainable adoption of CA practices. The study 143 has the objective of evaluating the agro-environmental aspects (i.e., soil C-related variables, 144 biological fertility, edaphic biodiversity, crop yield, and economic efficiency) in a direct 145 comparison between CA and CvtA systems on working farms where CA practices have been 146 147 implemented at different times.

## 148 2. Materials and methods

#### 149 **2.1. Study site and experimental design**

Twenty farms were chosen within the Po valley in 2013 with the first aim of evenly representing the 150 151 pedoclimatic conditions and cropping systems occurring in the area addressed by the HelpSoil project (Figure 1). The Po valley is a flat area and is approximatively 47000 km<sup>2</sup> wide; this area is 152 characterized by peculiar traits that make it unique for a variety of meteorological and agricultural 153 production systems variables (Fumagalli et al., 2013; Acutis et al., 2014). Climate is mild, the mid-154 latitude version of the Humid subtropical climate (Köppen climate classification Cfa and Cfb), and 155 156 farming systems are generally intensive with high N input (Perego et al., 2013). The farms designated for the present study were characterized by different production systems, a broad range 157 of soil properties, and critical issues to be addressed (e.g. low SOC content, weed pressure). Thus, 158 159 the majority of the Agricultural\_Systems under comparison were NT and CvtA, except for Cavallini, Rebollini, Euroagricola and Zanone farms (where only NT is present), Arisi (NT vs MT 160 vs CvtA), Carpaneta and Grandi farms (NT vs MT), Don Bosco and Cerutti (MT vs CvtA). Such an 161 162 experimental set up allows analyzing agro-environmental aspects under a wide range of technical and pedological conditions. 163

164 Over the course of the experiment, a varied range of meteorological conditions was recorded on the 20 farms involved in the project (Supplemental File 1); this aspect, along with the different 165 pedological characteristics, allowed us to capture a wide variety of conditions in which both CA and 166 CvtA practices were adopted. Mean annual rainfall from 2014 to 2016 varied from 709 mm 167 (Ruozzi) to 1645 mm (La Fattoria, Zanone). The 20 locations also differed in mean annual 168 temperature, which was the highest at Rossi (15.4 °C) and the lowest at Gli Ulivi (13.5 °C in 2016). 169 170 The monitoring on the demonstration farms was carried out from late 2013 to October 2016 (i.e. 32 months). The demonstration fields were set up on each farm with two or three replicates to compare 171 172 production and environment-related aspects under the farm current condition (i.e. CvtA, MT or NT) and the CA alternative option (MT or NT). The farms' key features, such as site location, soil 173 texture class, CA implementation year, crop rotation, and tillage treatments are listed in Table 2. 174 Further details on cover crops cultivation and soil characteristics are given in Supplemental File 2. 175

The chemical and physical parameters of soils were measured on soil sampled in early 2014. 176 Texture classes varied from sandy loam to clay; silty loam and silty clay loam were the soil types 177 most represented in the present study. A different code was assigned to the farms where CA was 178 implemented before 2006 (Long-term), from 2007 and 2013 (Medium-term), and at the beginning 179 of the experiment in 2013 (Short-term); this temporal distinction was chosen following the findings 180 of Pittelkow et al. (2015). Machinery with varied characteristics and brands were adopted for tillage 181 182 and sowing, according to the on-farm availability and the tested tillage treatments. In an attempt to picture real farm conditions, dates of tillage, sowing, harvesting, weed and pest control, and 183 irrigation were defined by local pedoclimatic conditions and farmers' experience. 184

185 Complete information about the 20 farms is given at the official project website
 186 *http://www.lifehelpsoil.eu/en/demonstrative-farms/.*

#### 187 **2.2. Data collection**

The collection of soil, crop, and field operation-related data was aimed at evaluating soil functionality along with the economic and technical feasibility of the tested treatments on the demonstrative farms. The following variables were measured at the beginning (March 2014) and at the end of the experiment (October 2016): SOC concentration and stock, soil bulk density, soil microbial carbon and respiration, the biological fertility index - BFI (Francaviglia et al., 2017), microarthropods, and the number of earthworms.

Prior to the data collection in 2014, soils were sampled in each experimental field to determine soil
texture, pH (in H<sub>2</sub>O and KCl) cation exchange capacity, magnesium, calcium, and carbonate (total
and active) content.

Soil samples (three independent replicates in each experimental field; 131 samples collected both in 2014 and in 2016) were collected at 0-0.3 m depth in each experimental field. The SOC content (g kg<sup>-1</sup>) was determined both on air-dry subsamples of 40-mg weight (0.5 mm sieved) using a ThermoQuest NA1500 elemental analyzer (Carlo Erba, Milano, Italy) and with the Springer-Klee

method (TOC) via oxidation with  $K_2Cr_2O_7$  and subsequent titration of unreduced  $Cr_2O_7^{-2}$  with Fe(NH<sub>4</sub>)<sub>2</sub>(SO<sub>4</sub>)<sup>2</sup>. The value measured by the former method was subtracted from the C-carbonate content, which was previously measured using a calcimeter. The latter method was employed as it is required for the estimation of the biological fertility index. In the present text, SOC content refers to the value estimated by the elemental analyzer.

The SOC stock was calculated from the SOC relative content and the bulk density measured on the same soil plot (three replicates at two soil depths, 0-0.15 m and 0.15-0.3 m in each experimental field; 262 samples collected both in 2014 and in 2016). The SOC stock (Mg) was estimated in the 0.3 m topsoil by using the formula [1] reported by Batjes et al. (1996):

where SOC (%) is the organic carbon content (%), *topsoil depth* is 0.3 m, BD is the topsoil bulk density (Mg m<sup>-3</sup>), and *RF* is the rock fragment content (%). BD was estimated on a known volume soil sample, which was collected with a cylindrical metal sampler pressed into the soil to both 0.15 and 0.3 m depths (three replicates). The sample was oven-dried at 105°C and then weighed; the value of bulk density was calculated as the ratio of oven-dried mass over the soil sample volume (g cm<sup>-1</sup>).

In an attempt to quantify the contribution of CA in SOC content and SOC stock, the data observed in CA in 2016 was related to those in CvtA for each implementation group, by applying a formula used by Powlson et al. (2014):

220 
$$SOCratio = (SOC_{CvtA} - SOC_{CA}) \times SOC_{CVTA}^{-1}$$
 [2]

221 
$$STOCKratio = (STOCK_{CvtA} - STOCK_{CA}) \times STOCK_{CVTA}^{-1}$$

These indices were multiplied by -1 so that the positive contribution of CA on SOC storage was indicated by a positive value.

224 Microbial biomass carbon (MBC,  $\mu g g^{-1}$  dry soil) was estimated via fumigation extraction, 225 following the procedure detailed by Francaviglia et al. (2017). The cumulative microbial respiration 226 was determined over 28 days of incubation by measuring the CO<sub>2</sub> emitted from a 20-g moist sample after 1, 3, 7, 10, 14, and 21 days. The cumulated CO<sub>2</sub> emitted from 21 to 28 days of incubation represented the basal respiration value. The following parameters and indices, which were derived from TOC and microbial-related measurements, were taken into account to calculate the BFI: organic matter OM (TOC × 1.72), MBC ( $\mu$ g g<sup>-1</sup> dry soil), cumulative respiration (mg CO<sub>2</sub>-C h<sup>-1</sup> kg<sup>-1</sup> soil), basal respiration (mg CO<sub>2</sub>-C h<sup>-1</sup> kg<sup>-1</sup> soil). The metabolic and mineralization quotients were calculated as follows:

233 metabolic quotient= basal respiration × 
$$MBC^{-1} \times 10^3 (\mu g h^{-1} \mu g^{-1})$$
 [3]

234 mineralization quotient = cumulative respiration 
$$\times TOC^{-1}$$
 (µg µg<sup>-1</sup>) [4]

For each of these parameters and indices, a score (1 to 5) was assigned; score ranges related to each parameter were derived from Francaviglia et al. (2017) and are given in Table 3. The BFI was calculated by adding up the scores obtained for the five microbial indices.

As for the microarthropods, the procedure was carried out by applying the method reported by 238 Tabaglio et al. (2009a). Accordingly, an undisturbed soil core ( $10 \text{ cm} \times 10 \text{ cm} \times \text{cm}$ ) was sampled; 239 microarthropods were extracted from each core using a Berlese-Tullgren funnel and then stored in a 240 storage solution (i.e. 70% industrial ethylic alcohol and 5% glycerol). The microarthropods were 241 counted using a microscope and soil biological quality was expressed using the QBS-ar index 242 (Parisi et al., 2005). The QBS-ar index is based on the assumption that the richer is the well-adapted 243 244 microarthropod community, the higher the soil quality. The degree of soil adaptation of each microarthropod group is defined by assigning an eco-morphological score (EMS), ranging from 1 to 245 20, according to the morphological traits that are suitable for the edaphic habitat (e.g., 246 anophthalmia, depigmentation, and reduction of appendices). The OBS-ar is the numerical value 247 (generally between 20 and 280) resulting from the sum of the EMS indexes assigned to each 248 taxonomic group of microarthropods. 249

The number of earthworms was counted on 15625 cm<sup>3</sup> (25 cm  $\times$  25 cm  $\times$  25 cm) undisturbed soil samples (FAO, 2008). Also, for both the tested treatments on each farm, the aboveground biomass and crops grain yield were estimated by weighing the plants harvested on three areas of 5 m<sup>2</sup> in each experimental field and then pooled together. The biomass and grain dry matter content were obtained by weighing a 1kg biomass subsample after 24 h at  $105^{\circ}$ C in a dry oven.

An index proposed by West et al. (2010), namely the tradeoff index, was calculated here to relate the SOC difference between CvtA to CA to the difference in C-biomass yield between CvtA to CA (C-yield<sub>CA</sub> and C-yield<sub>CvtA</sub>), which was observed over the experiment on each farm. The tradeoff index was calculated as follows:

260 Tradeoff index = 
$$(SOC_{CA} - SOC_{CvtA}) \times (C-yield_{CA} - C-yield_{CvtA})^{-1}$$
 [5]

Where SOC<sub>CA</sub> and SOC<sub>CvtA</sub> are SOC content under CA and CvtA, respectively. The index was calculated for 16 farms as in four farms (i.e., Cavallini, Euroagricola, Rebollini, and Zanone) NT was the only examined treatment.

Fuel consumption and human labor were taken into account in the calculation of a simplified energy and economic balance, which was performed by following the approach proposed by Grisso et al. (2004) on data collected in the farms. Five farms were chosen out of the 20 farms involved in the experiment to compare the effect of CA and CvtA treatments.

Fuel consumption (1  $ha^{-1}$ ) required in field operations (i.e., soil tillage, seeding, weed control, fertilization, irrigation, and harvesting) was estimated based on the number of operations, the tractor power, and the time required per operation (h  $ha^{-1}$ ). The calculation was performed as follows:

271 
$$Q = (0.22 \times X + 0.096) \times (1 - (-0.0045 \times X \times N_{red} + 0.00877 \times N_{red})) \times P_{PTO} \times h$$
 [6]

where Q = diesel fuel consumption, (1 ha<sup>-1</sup>), N<sub>red</sub> = the percentage of reduced engine speed for a partial load from full throttle (%), X = the ratio of equivalent PTO power to rated PTO power,  $P_{pto}$  = the rated PTO power, kW, and h=operation time (h ha<sup>-1</sup>). Values were derived from machinery technical data sheets. The analysis also took into account the information given by the five farmers about the material purchased for crop grpwing (e.g., seeds, mineral fertilizers, and agrochemicals) and the labor hours required for each operation; details are reported in Supplemental File 3.

The account of direct costs and gross crop yield enabled the calculation of the gross economic gain (i.e., revenue from grain yield subtracted by direct costs) and the ratio of the revenue from grain yield over direct costs, which is a dimensionless index that quantifies the efficiency of the tested treatments.

# 283 **2.3. Statistical analysis**

Effects of Agriculture\_Systems (CA vs CvtA), Implementation\_Year, and Farm were tested on 284 SOC relative content, SOC stock, bulk density, MBC, microbial cumulative and basal respiration, 285 BFI, QBS-ar, and earthworms using a repeated measure mixed model with unstructured matrix 286 (IBM – SPSS 25, IBM Corporation, Armonk, New York, US). The Farm factor was nested within 287 the CA implementation year factor (Implementation Year). The levels of the factor 288 Implementation\_Year were three, namely Long-term (before 2006), Medium-term (from 2006 to 289 2013), and Short-term (after 2013). Repeated measures were used as two samplings were carried 290 out in the same field site in 2014 and in 2016. The mixed model was applied to the data collected 291 under the tested treatments on the 14 farms for which an actual comparison were set between CA 292 and CvtA (see details on treatments tested on each farm in Table 2). The Sidak post hoc test was 293 applied for means comparison. 294

The relationship between the C-related variables was tested using linear and non-linear regression to study the trend in microbial respiration affected by the SOC content.

The linear mixed model was run by splitting the dataset by crop to investigate the effect of Agriculture\_Systems and Implementation\_Year on the grain production of the most common crops, namely maize, soybean, and winter wheat. For the other crops, less than two replicates across farms were available and, in turn, the related results were assumed to be insufficient for testing the effectof the Agriculture\_System.

After checking for normality and homogeneity of variances, a one-way ANOVA was run to test the effect of the factor Year on the SOC tradeoff index, which is the ratio of SOC variation from CvtA to CA (NT or MT, according to the farm management) over the C-yield variation from CvtA to CA. As for the simplified economic balance, a one-way ANOVA model was applied to data of oil consumption for field operation, costs for raw material purchase, labor hours, total costs, the gross economic gain (i.e., revenue subtracted by costs), and the ratio of revenue over costs to find differences between NT and CvtA (NT vs MT in Carpaneta).

## 309 **3. Results**

In comparison with CvtA, overall positive effects of CA were found in the following farms: Arisi, Cerzoo, La Fattoria, Mosca, Rossi, Ruozzi, Sant'llario, and Sasse Rami. No relevant differences were observed in Cerutti, Don Bosco, Gli Ulivi, Pasti, and Vallevecchia; negative effects of CA were not found. The results of SOC content and stock, C-microbial biomass related variables, microarthropods, earthworms, yield, carbon tradeoff index, gross gain, and economic efficiency index are listed below.

## 316 **3.1. Carbon-related variables**

Regarding the application of the mixed model, the between-groups effect of the Farm X 317 Agricultural\_Systems was significant for SOC content (measured via both the methods applied, 318 Elemental Analyzer, and Springer-Klee), SOC stock, C-microbial biomass, cumulative respiration, 319 320 and the BFI (Table 4). Farms where a significantly higher SOC content (Elemental Analyzer) was found in CA than in CvtA were: Arisi (17.9 and 16 g kg<sup>-1</sup>, +11%), Cerzoo (15.2 and 13 g kg<sup>-1</sup>, 321 +17%), Sant'Ilario (21.6 and 18.7 g kg<sup>-1</sup>), and Sasse Rami (17.9 and 12.5 g kg<sup>-1</sup>, +43%). 322 Conversely, CvtA was found to have slightly higher SOC content in La Fattoria (20 and 18.2 g kg<sup>-1</sup>, 323 +10%). The results of the analysis performed on TOC were consistent with those of SOC content. 324

The analysis also showed a significant effect of the interaction between Agricultural Systems and 325 Implementation Year on SOC content and BFI. Overall, the significant differences between CA 326 and CvtA were found for the Medium-term group (Table 5). The first run of the linear mixed model 327 highlighted a non-significant effect of the sampling depth on bulk density and consequently the 328 mixed model was applied to the mean bulk density data, which were calculated as the mean 329 between the two layers for each experimental field. The CvtA resulted in significantly higher bulk 330 density than CA for the medium-term group. Farms, where BD in CvtA was higher than in CA, 331 were: Cerzoo (1.56 and 1.46 Mg m<sup>-3</sup>), Diana (1.37 and 1.25 Mg m<sup>-3</sup>), Pasti (1.25 and 1.16 Mg m<sup>-3</sup>), 332 Sant'Ilario (1.26 and 1.13 Mg m<sup>-3</sup>) and Sasse Rami (1.23 and 1.12 Mg m<sup>-3</sup>). Similarly to the SOC 333 334 content, SOC stock was significantly higher in CA than in CvtA for the Medium-term group. As for the significant interaction between farms and Implementation\_Year, in two Medium-term and 335 Short-term farms (i.e. Sasse Rami and Ruozzi), CA determined higher SOC stock in comparison 336 337 with CvtA. A positive and significant contribution of CvtA in SOC stock was not found in any farm. Considering all of the collected data, including those on the farms where only CA was 338 adopted, SOC content and stock were the highest in the Long-term group (Figure 2a, 2b). 339

The SOCratio and STOCKratio are shown in Figure 3. The displayed bars are the confidence interval at 95%; bars crossing the zero point indicate that the SOC content and stock under CA does not differ from the one in CvtA. In agreement with the statistical analysis results, the Medium-term group had a more significant effect of CA on SOC concentration and stock than in CvtA.

The CvtA resulted in a lower MBC within the Medium-term group, while it was higher, even if not significantly, for the Short-term group (Table 5). MBC was significantly higher in CvtA than in CA only in Cerutti (299 and 193  $\mu$ g g<sup>-1</sup>); in Cerzoo, Diana, Pasti, Sant'Ilario, Sasse Rami, and Vallevecchia, CA's MCB was double that of CvtA's (on average, 236 and 119  $\mu$ g g<sup>-1</sup>). Regarding the effect of tillage, higher MCB across Implementation\_year was generally observed in MT (Figure 2c).

Basal respiration was affected significantly by Agricultural\_Systems and Implementation\_year 350 (Table 4). In particular, the lowest values were found in CA and for the Short-term group (5 mg 351 CO<sub>2</sub>-C h<sup>-1</sup> kg<sup>-1</sup> soil) compared with the Long-term (6.3 mg CO<sub>2</sub>-C h<sup>-1</sup> kg<sup>-1</sup> soil) and the Medium-352 term (6.7 mg CO<sub>2</sub>-C  $h^{-1}$  kg<sup>-1</sup> soil) groups. The Agricultural Systems × Implementation year 353 interaction was found significant for the cumulative respiration (i.e., higher in CA × Medium-term 354 group). In two farms, the cumulative respiration was higher in CA than in CvtA (Sant'Ilario, 393 355 and 285 mg CO<sub>2</sub>-C h<sup>-1</sup> kg<sup>-1</sup> soil; Vallevecchia: 395 and 237 mg CO<sub>2</sub>-C h<sup>-1</sup> kg<sup>-1</sup> soil). The metabolic 356 and mineralization quotients had a trend similar to that of TOC and cumulative respiration. 357

A positive linear correlation was detected between TOC and cumulative microbial respiration data collected in 2016 (Figure 4). At a SOC value higher than approximatively 18 g kg<sup>-1</sup>, cumulative soil respiration rose steeply and in turn reduced the amount of the sequestered SOC.

BFI results were the highest under CA in the Medium-term group (Table 4). As for the tillage practices, BFI was higher in NT and MT compared to CvtA within the Long-term and Mediumterm groups in 2016 (Figure 2d). Moreover, while distinguishing the evolution of the BFI by classes, Agricultural\_Systems and Implementation\_Year (Figure 5), the most relevant improvement in classes was found under NT and MT for the groups B and C, for which CvtA resulted in lower classes in 2016 than in 2014.

## 367 **3.2. Microarthropods and earthworms**

Soil biodiversity was assessed by estimating the QBS-ar index of the 0.1 m topsoil and the number of earthworms present in the topsoil layer (0-0.25 m) in the tested treatments of the 20 farms. The highest results of QBS-ar were observed in the Medium-term group under CA, while the number of earthworms generally decreased from Long to Short-term groups (Table 5). The farms where QBSar was significantly lower in CvtA than in CA were Cerzoo (51.5 and 100.2, respectively), Pasti (86.3 and 63.8, respectively), and Sasse Rami (52.3 and 12.8, respectively). Regarding tillage practices, QBS-ar was generally similar between MT and NT and lower in CvtA, except for the Medium-term group. The QBS-ar index was found the highest in MT for the Medium-term Implementation\_Year group (141); in CvtA and in NT the index was equal to 63 and 83, respectively (Figure 2e).

CA resulted in higher earthworm abundance compared to CvtA in Cerzoo (21.2 and 2.3), Diana (27 and 8.3), La Fattoria (17.7 and 7.3), Pasti (30 and 5), and Ruozzi (13.7 and 7). Considering the whole dataset split by tillage practices, the mean number of earthworms was the highest for the Long-term group in NT (24.3) (Figure 2f). Generally, earthworms were less abundant in CvtA than in NT.

## **383 3.3. Grain yield**

The effect of the Agricultural\_Systems was found close to the significance threshold (p=0.07) in maize and significant in soybean (p=0.01) (Table 6). The grain yield of maize, winter wheat, and soybean in CA and CvtA are displayed split by Implementation\_Year (Long-term, Medium-term, Short-term) in Figure 6. Maize yield in CvtA was 15% higher than in CA across the Implementation\_Year groups. Soybean grain yield was significantly lower in CA than in CvtA, with a lower difference in the Long-term group (-11%) than in Medium-term (-17%) and Short-term (-20%) groups. No significant difference in winter wheat yield was found between CvtA and CA.

The tradeoff index (Mg C-soil  $ha^{-1}/Mg$  C-crop yield  $ha^{-1} y^{-1}$ ) was calculated as the ratio of the SOC 391 variation over the C-biomass yield variation between NT and CvtA with the aim of simultaneously 392 393 considering the effect of the Agricultural\_System on production and environment-related aspects. The tradeoff index varied significantly between farms (Figure 7). For eight farms, the index was 394 negative as the increase in soil C stock offset the negative variation of C-yield production; these 395 396 farms belonged to the Medium-term and Short-term Implementation\_Year groups. A decrease in SOC stock and negative variation of C-yield production was estimated for five farms with no effect 397 of Implementation Year. An increase in SOC stock and a positive variation of C-yield production 398 was calculated in the remaining four farms, which belong to the Short-term group (Arisi, 399

index=2.05), the Medium-term group (Rossi, index=1.85), and the Long-term group (Grandi,
index=2.57; La Fattoria, index=3.94).

#### 402 **3.4.** Costs and fuel consumption

A simplified economic balance was calculated to compare the gross gain (i.e., revenue from grain 403 404 yield subtracted by direct costs) and the economic efficiency (i.e., the ratio of revenue over direct costs) for the two treatments under comparison on five farms involved in the present study (i.e., 405 Carpaneta, La Fattoria, Mosca, Pasti, and Ruozzi). Production costs and revenue from grain yield, 406 407 and in turn gross margin and economic efficiency, were different between years and farms and varied according to field operation, fuel consumption, labor, and raw material purchase (e.g., seeds 408 and agrochemicals). The statistical analysis carried out to compare the costs of the operations 409 410 performed in both the treatments (e.g., application of slurry, sowing, and harvesting) did not show any significant differences between the two Agricultural Systems under comparison on each farm. 411 Considering the annual costs for the two Agricultural Systems under comparison, the fuel 412 consumption was generally lower in NT than in CvtA; in Carpaneta, MT and NT did not differ 413 except in the first year when the cover crop was grown only in NT. The fuel consumption was 414 significantly higher in CvtA compared to NT in Ruozzi in the first two cropping seasons and in 415 Mosca and La Fattoria over the three years. In Carpaneta, the fuel consumption was significantly 416 higher in NT than in MT in the first cropping season and that was due to the cultivation of the crop 417 cover (i.e., crop mixture of vetch, rye, and radish), while in the third year the consumption in MT 418 was higher than in NT due to the vertical tillage machine (i.e., disc harrow). Labor hours did not 419 significantly differ between treatments. 420

Among the field operations, weeding in NT was by approximatively 30% more expensive than in the comparative treatment. Fuel consumption for soil tillage accounted for 40% of total consumption (i.e. the 36% of total costs due to field operations) in CvtA while it was zero in NT. 424 The fuel consumption and total cost of the sod-seeding in NT (on average, 9 L ha<sup>-1</sup> and 20  $\in$  ha<sup>-1</sup>) 425 did not differ to those related to the seeding in CvtA (on average, 10 L ha<sup>-1</sup> and 23  $\in$  ha<sup>-1</sup>).

Considering the raw material purchase, the difference between NT and MT in Carpaneta in 2013-426 2014 was mainly due to the cover crops' seeds purchase (approximatively 25%). In La Fattoria the 427 winter cover crop was barley, and the seeds costs represented only 3% of the annual costs for 428 material purchase. Overall, the cost for herbicides' purchase was 18% more expensive in NT than in 429 430 the comparative treatment, whereas the fuel consumption and total costs for weeding operations did not differ between NT and CvtA. The difference consisted in a higher number of weeding operation 431 (1 to 2), which was due to the termination of the cover crops (in Carpaneta, Pasti, and Ruozzi) and 432 433 to an additional intervention aimed to control a higher pressure of weeds (in Pasti and Ruozzi).

434 Crop yields, the gross margin and the indicator of economic efficiency for the treatments under 435 comparison on the five examined farms are displayed in Table 7. Variation in gross gain was 436 mainly driven by the differences in grain yield between treatments, which was generally lower in 437 NT than in the comparative treatment (-0.5 Mg DM ha<sup>-1</sup>). Consequently, the gross gain was 438 generally lower in NT; conversely, the economic efficiency index was comparable and even higher 439 in NT in La Fattoria.

# 440 **4. Discussion**

#### 441 *4.1 Soil-related variables*

In the literature, findings are reported to support the capability of CA to result in positive changes in SOC content and stock compared to CvtA (Govaerts et al., 2009; Ogle et al., 2012; Virto et al., 2012; Abdalla et al., 2013; Stavi et al. 2016). The statistical analysis highlighted the significant effect of Implementation\_Year × Agricultural\_Systems on SOC storage, namely concentration and stock. CA within the Long-term and Short-term groups did not result in significantly higher SOC content and SOC stock (Table 5). However, this result did not inform about the gain in SOC storage that is achievable in CA compared to CvtA. The data of SOCratio and STOCKratio suggest that a

significant SOC gain occurred for the three groups, especially for the Medium-term (Figure 3). This 449 450 result agreed with the data reported by Tabaglio et al. (2008; 2009a; 2009b) from four trials in the Po valley. The SOCratio estimated in this study for the 0.3 m topsoil was higher than that shown by 451 452 Powlson et al. (2014). The low results of the SOCratio and STOCKratio of the Long-term group was likely due to the low number of cases, and this is because farms where the implementation year 453 of CA is before 2006 are unlike to allocate fields to CvtA practices. Also, in one farm belonging to 454 455 the Long-term group (i.e. Pasti), tillage in CvtA consisted of moderate plowing, as confirmed by the low field operation costs (Table 7). Nonetheless, considering data collected under NT, also on farms 456 where CvtA practices were not performed, the long-term contribution of NT on SOC content and 457 458 stock is evident (Figure 2a and 2b).

In the present study, the BFI index was calculated to assess the effect of the agricultural systems on 459 soil biological fertility. The calculation of the BFI enabled the evaluation of the overall biological 460 461 status of the examined soils (Renzi et al., 2017), as it is effectively used as a multi-domain indicator of biological fertility. NT and MT soils within the Long-term and Short-term Implementation\_Year 462 groups scored the highest value, which was similar to the values found by Francaviglia et al. (2017) 463 for cork oak forest (16-20). The values observed in CvtA approached the range found in pastures 464 (11-13) by Francaviglia et al. (2017). Moreover, the most evident improvement in the BFI ranking, 465 namely from average to the high class, was observed in the Medium-term and Short-term 466 Implementation Year groups. 467

The QBS-ar index is based on the assumption that the richer is the well-adapted microarthropod community, the higher the soil quality, the QBS-ar was 18% lower in CvtA than in NT. Average values were in agreement with the value found by Menta et al. (2018) for agricultural soils. Tabaglio et al. (2009) reported higher values of QBS-ar in no-till soils compared to conventional tillage soils. In this study, The QBS-ar value was the highest in CA, especially under MT, within the Medium-term Implementation\_Year group. The time of implementation of a certain Agricultural\_System might result in a difference in QBS-ar; Simoni et al. (2013) found higher

QBS-ar values in a recent organic maize-based agroecosystem (6 years) than in an old one (16 475 476 years). Similarly, we found the highest QBS-ar value for the Medium-term Implementation Year group (2007-2013) for all the Agricultural\_Systems. A significant effect of the type of 477 Agricultural\_Systems was also observed on the number of earthworms for the Long-term and the 478 Medium-term groups. This outcome is in agreement with Triplett and Dick (2008) who found and 479 abundance of earthworms on fields having long implemented CA practices. In CA, the abundant 480 481 surface mulch, which is due to cover crops and reduced tillage, provides food, nutrients and energy for earthworms, arthropods, and micro-organisms below ground that also biologically till soils 482 (Hobbs et al., 2008; Ranaivoson et al., 2017). Van Cappelle et al. (2012) found that the CA 483 484 practises resulted in significantly higher earthworm abundance than CvtA in silty and loamy soils. This result agrees with the results of the present study, in which the farms where a significant effect 485 of CA on the earthworm abundance was detected had sandy clay loam to clay soils. 486

# 487 *4.2 Crop yield and tradeoff index*

The general decrease in crop yield passing from CvtA to CA indicated by various authors (Ogle et 488 al. 2012; Scopel et al., 2013; Powlson et al., 2014) was particularly evident only for the Short-term 489 490 group. Conversely, the lower variation in C-biomass from CvtA to CA production was observed in farms of the Long-term and Medium-term groups, especially in winter wheat. Van den Putte et al. 491 (2010) suggested lessening the decrease in winter cereal yield under NT by adopting a multi-crop 492 493 rotation, as it is a fundamental principle of the CA to make it a viable solution for farmers. This practice was adopted on the farms investigated in this study. This evidence also arose from the 494 495 meta-analysis performed by Pittelkow et al. (2015), in which decreases in crop yield passing from CvtA to NT do not occur only in the case of residues retained on soil and multi-crop rotations. An 496 497 outcome of this study was that the dispersion of the data set was higher in CA than in CvtA as it 498 was less stable across locations. This fact was likely due to the effect of the pedoclimatic conditions and the different skills of the farmers. 499

The tradeoff index proposed by West et al. (2010) was defined under the assumption that CA 500 always results in yield decrease and SOC gain. The outcome of the present study was 501 heterogeneous, as CA did not regularly result in higher SOC stock and concurrent yield losses. 502 503 Results of farms where the CA was implemented as of 2006 (i.e. Medium-term and Short-term groups) indicated that the SOC stock variation from CA to CvtA was positive, while yields 504 decreased. The Long-term group resulted in null to positive results. In the other two farms of the 505 Long-term group (i.e., Grandi and La Fattoria), positive values indicated a higher SOC stock 506 variation in CA than in CvtA and concurrent higher yields. 507

#### 508 *4.3 Costs and energy balance*

Overall, the gross gain was higher in the comparative thesis (CvtA in four of the five tested farms), while the indicator of economic efficiency suggests the opportunity of using conservative farming practices, especially in paddy rice production and in farms in which the introduction of conservative practices is before 2006. The overall reduction of costs and the increase in economic balance was also reported by Hobbs et al. (2008) and Scopel et al. (2013). However, in the comparison of the economic balance between CA and CvtA, some aspects might result in a reduced gain in economic efficiency.

In Carpaneta, the management of cover crop during the first cropping season (2013-2014) and the significantly lower production of maize caused a decrease in the indicator of economic efficiency in NT compared to MT. However, in NT compared to MT there is a higher content of organic carbon (+ 0.2%) and a higher final BFI. In a Long-term perspective, a solution aimed to reduce the cost of cover crop seeds might be to use the grain of cereals produced on-farm (e.g., rye and wheat), in rotation with purchased seeds (e.g., mustards, vetch, black oat, and tillage radish).

The opportunity of using on-farm seeds for enhancing the economic sustainability has been not investigated and would offer a new solution in the cropping system designing. Generally, the most critical aspect, which is also the most relevant environmental issue concerning CA, is the costs

associated with the purchase of herbicides, which was higher in NT than in CvtA. Weeds possibly 525 reduce crop yield while increasing management costs. Weeds control was referred as critical by 526 many authors (Scopel et al., 2013; Powlson et al., 2014; Stavi et al., 2016) since the economic 527 advantage due to the reduced tillage might be counterbalanced by higher costs for herbicides 528 purchase and spraying. Tabaglio and Gavazzi et al., 2009, reported a decrease in weed pressure in a 529 NT system after three years since the implementation in a maize-based system in the Po valley. 530 Another option could be to couple optical sensors to global positioning system information enable 531 to distinguish weeds from crops and in turn perform precision weed control (Westwood et al., 532 2018), using a lower rate of herbicides. 533

# 534 **5. Conclusions**

The monitoring carried out in 2014 and in 2016 on 20 farms allowed not only to compare different management approaches of the crop systems but also to evaluate the evolution in short to medium term of the agro-environment aspect under examination. The results obtained in the present study indicate the environmental and economic sustainability of the agronomic management adopted in the different pedoclimatic conditions experienced on the 20 farms.

The comparison of the variables did not show a common trend between the farms as they represent 540 unique realities, located in different areas resulting in heterogeneity of the adopted practices. The 541 farms where conservation agriculture practices resulted in positive results of both soil fertility and 542 economic efficiency are those in which such practices were implemented long before the beginning 543 of the present study. Consequently, the optimization of the practices requires the acquisition of 544 knowledge and the development of technical skills. On farms in which conservation agriculture 545 practices were adopted after 2006, conservation agriculture was found to increase the percentage of 546 organic carbon content and the biological fertility index, with a higher extent than the conventional 547 systems, although grain yield was significantly lower by 15% on average. 548

Our results suggest that the adoption of conservation agriculture practices is feasible in the Po 549 550 valley environment. After an initial phase required for farmers to develop technical skills, it is possible to reduce the yield gap between conservation and conventional systems. Policy support for 551 technical training in CA is needed and should be reinforced at farm and district scales. Conversely, 552 the lack of a technical support might result in the abandoning of CA practices with a return to 553 conventional systems after the end of the 2014-2020 European subsidies program. Moreover, 554 involving farmers with the participatory approach in defining the strategies to adopt at field and 555 farm scale is regarded as fundamental for the effectiveness of their implementation (Nguyen et al., 556 2016; Schindler et. al., 2016). As already suggested by Pradhan et al. (2017), institutionalizing CA 557 558 into regional institutions will enhance the sustainability of the technology. A side effect of the present study was to connect farmers, even from different areas of the wide Po plain by adopting a 559 participatory approach involving scientists, technical professionals from both public agencies and 560 561 private sector, and policy makers.

## 562 Acknowledgments

The authors gratefully acknowledge funding from the European Union to the LIFE+ project 563 HelpSoil LIFE12 ENV/IT/000578 and Kunh S.r.l. Italy. The authors thank Regione Lombardia 564 (leading partner), Regione Piemonte, Regione Emilia-Romagna, Regione Veneto, Regione Friuli 565 Venezia Giulia, ERSAF, Vento Agricoltura, and C.R.P.A. S.p.A (partners beneficiaries). The 566 authors acknowledge researchers (namely Luisella Celi, Dario Sacco, Barbara Moretti, Dario Sacco 567 from the University of Turin; Francesco Morari from University of Padua), field technicians, and 568 the farmers involved in the project for the helpful suggestions and the productive discussions. The 569 570 HelpSoil project has been recently awarded by the Euroepan Commission as one of the 12 Best LIFE-Climate action Projects 2016-2017 (http://ec.europa.eu/environment/life/bestprojects/bestclim16-571 572 17/index.htm).

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# Tables

Table 1. Recent published reviews (2007-2017) concerning agro-environmental aspects related to the implementation of conservation tillage and cover crops in temperate areas. A synthetic result was retrieved from each review. The symbols '+', '-', and '=' indicate advantage, negative effect, and similar effect of conservation agriculture (CA) practices than the conventional agriculture (CvtA), respectively. Reviews on the same row had similar results of conservation tillage and cover crops effect. Some reviews dealt both with conservation tillage and cover crops; in this case two scores are assigned.

	Conservation agri	culture in compari	son with conventional tillage
	Conservation Tillage	Cover Crops	
Yield	= / -	=	Knowler and Bradshaw, 2007; Hobbs et al., 2008; Van den Putte et al., 2010; Stavi et al. 2016; Ranaivoson et al., 2017
	-		Ogle et al., 2012; Scopel et al., 2013; Powlson et al., 2014
		=	Blanco-Canqui et al., 2015
	-	+	Pittelkow et al., 2015
	+/-		Farooq et al., 2011
		+	Delgado et al., 2007
Farm management	+		Hobbs et al., 2008; Scopel et al., 2013
	+/-		Knowler and Bradshaw, 2007; Powlson et al., 2014; Ranaivoson et al., 2017
Weed management	+/-		Armengot et al. 2015; Farooq et al., 2011
	-		Knowler and Bradshaw, 2007; Powlson et al., 2014; Ranaivoson et al., 2017; Stavi et al. 2016
Soil structure	+		Hobbs et al., 2008; Triplett and Dick, 2008; Morris et al., 2010; Abdalla et al., 2013; Powlson et al., 2014
		+	Blanco-Canqui et al., 2015
Nutrient use efficiency	+		Morris et al., 2010; Delgado et al., 2007
	+/-	+	Scopel et al., 2013
		+	Quemada et al., 2013
Moisture retention/infiltration	+	+	Knowler and Bradshaw, 2007; Scopel et al., 2013
		+	Delgado et al., 2007; Blanco-Canqui et al., 2015
	+		Palm et al., 2014; Stavi et al. 2016
	+ / =		Ranaivoson et al., 2017
Soil temperature	+	+	Hobbs et al., 2008; Triplett and Dick, 2008; Morris et al., 2010; Scopel et al., 2013; Lal, 2015
Soil C sequestration	+		Virto et al., 2012; Abdalla et al., 2013; Stavi et al. 2016
	=		Powlson et al., 2016
	-		Ogle et al., 2012
		+	Delgado et al., 2007; Blanco-Canqui et al., 2015; Lal, 2015
	+ / =	+	Aguilera et al., 2013
	=	+ / =	Palm et al., 2014
	+ / =		Ranaivoson et al., 2017
	+ / = / -		Govaerts et al., 2009
Effect on GHGs emissions	+ / =		Palm et al., 2013; Abdalla et al., 2013
	=		Powlson et al., 2014
	+		Morris et al., 2010; Kaye and Quemada, 2017
Soil Biodiversity	+	+	Knowler and Bradshaw, 2007; Van Capelle et al., 2012; Powlson et al., 2014
	+		Triplett and Dick, 2008
Fuel consumption	+		Knowler and Bradshaw, 2007; Morris et al., 2010; Scopel et al., 2013; Powlson et al., 2014
Economic balance	+/-		Knowler and Bradshaw, 2007; Pannell et al., 2014
		+/-	Blanco-Canqui et al., 2015
	+ / =		Scopel et al., 2013
	+		Hobbs et al., 2008; Triplett and Dick, 2008

Table 2. Overall features of the farms involved in the present study. Conservation agriculture (CA) was implemented before 2006 (=Long-term), between 2006 and 2013 (=Medium-term), after 2013 (=Short-term). The CA agricultural system was NT (with exception of Cerutti and Don Bosco) and included the cultivation of winter cover crops. CA implied the cultivation of cover crops, except for the farms in Piedmont. CvtA=conventional system. CvtA=conventional system, MT=minimum tillage, NT=no-till.

	Administative Italian Region	Farm	Location	Soil type	Year of CA implementation	Tested treatments	(	Crop rotation (2013-20	016)	
				•	*		2013-2014	2014-2015	2015-2016	
	Emilia-Romagna	Cerzoo	45.01 N 9.71 E	Silty clay loam	2010 Medium-term	NT , CvtA	maize	soybean	w.wheat	
		Gli Ulivi	44.13 N 11.92 E	Clay	2015 Short-term	NT , CvtA	lucerne	w.wheat	sorghum	
		Ruozzi	44.71 N 10.77 E	Clay	2013 Short-term	NT , CvtA	w.wheat	maize	w.wheat	
	Friuli Venezia Giulia	La Fattoria	46.05 N 13.35 E	Loam	2006 Long-term	NT , CvtA	maize	soybean	barley - soybean	
	Lombardy	Arisi	45.15 N 10.16 E	Silt loam	2014 Short-term	NT , MT	maize	maize	soybean	
						CvtA	maize	maize	maize	
vtA		Rossi	45.14 N 10.11 E	Silt loam	2010 Medium-term	NT	maize	w.wheat - soybean	w.wheat	
ç						CvtA	maize	maize	maize	
A v	Piedmont	Cerutti	44.94 N 7.90 E	Silt loam	2014 Short-term	MT , CvtA	maize	maize	maize	
Ŭ		Don Bosco	44.88 N 7.69 E	Sandy loam	2006 Long-term	MT , CvtA	maize	w.wheat	maize	
		Mosca	45.22 N 8.13 E	Sandy loam	2013 Short-term	NT, CvtA	rice	rice	rice	
	Veneto	Diana	45.58 N 12.30 E	Loam	2009 Medium-term	NT , CvtA	maize	soybean	w.wheat	
		Pasti	45.56 N 12.76 E	Sandy clay loam	2005 Long-term	NT , CvtA	barley - soybean	maize	soybean	
		Sant'Ilario	45.40 N 12.15 E	Sandy clay loam	2008 Medium-term	NT , CvtA	maize	soybean	w.wheat	
		Sasse Rami	45.05 N 11.89 E	Loam	2008 Medium-term	NT , CvtA	maize	soybean	w.wheat	
		Vallevecchia	45.63 N 12.94 E	Sandy loam	2008 Medium-term	NT , CvtA	maize	soybean	w.wheat	
	Emilia-Romagna	Cavallini	44.67 N 12.76 E	Clay loam	2008 Medium-term	NT	w.wheat	soybean	w.wheat	
						NT	w.wheat	soybean	soybean	
NT	Friuli Venezia Giulia	Euroagricola	45.87 N 13.02 E	Silty clay loam	1998 Long-term	NT , NT	soybean	w.wheat	maize	
IT,		Zanone	46.10 N 13.43 E	Silt loam	2010 Medium-term	NT , NT	sorghum	soybean	sorghum + soybean	
N.	Lombardy	Carpaneta	45.19 N 10.88 E	Clay loam	2012 Medium-term	NT , MT	soybean	maize	w.wheat $+$ clover	
CF		Grandi	45.09 N 9.20 E	Silty clay loam	2003 Long-term	NT , MT	maize	w.wheat	maize	
		Rebollini	44.96 N 9.18 E	Loam	2015 Short-term	NT	lucerne	barley	buckwheat	
						NT	lucerne	barley	lucerne	

Table 3. Scores of the intervals of values for the different parameters and classes of the biological fertility index (BFI). TOC=soil organic carbon content (g kg<sup>-1</sup>) estimated with the Springer-Klee method.

			S	core range	s	
Parameter (or index)	Unit of measurement	1	2	3	4	5
Organic matter *	g kg <sup>-1</sup>	<1	1-1.5	1.5-2	2-3	>3
Microbial organic carbon (MBC)	$\mu g g^{-1}$	<100	100-200	200-300	300-400	>400
Basal microbial respiration	mg $CO_2$ -C h <sup>-1</sup> kg <sup>-1</sup> soil	<5	5-10	10-15	15-20	>20
Cumulative microbial respiration	mg $CO_2$ -C h <sup>-1</sup> kg <sup>-1</sup> soil	<100	100-250	250-400	400-600	>600
Metabolic quotient	$\mu g \operatorname{CO}_2 - \operatorname{C}_{\text{basal}} \operatorname{h}^{-1} \mu g^{-1} \operatorname{MBC}$	>0.4	0.3-0.4	0.2-0.3	0.1-0.2	< 0.1
Mineralization quotient	$\mu g CO_2 - C_{cumulative} \mu g^{-1} TOC$	<1	1-2	2-3	3-4	>4
Biological Fertility index	-	Ι	Π	III	IV	V
		0-6	6-12	13-18	19-24	25-30
		very low	low	average	good	high

\*Organic matter was calculated by multiplying TOC g kg-1 by 1.72

Table 4. Results of the linear mixed model applied to test the effects of the Agricultural\_Systems (CA vs. CvtA) on the carbon-related variables, bulk density, biological fertility index, QBS-a, and earthworms count by using a repeated measure mixed model where the farm factor was nested within the Implementation\_Year factor (Long-term=before 2006, Medium-term=from 2006 to 2013, Short-term=after 2013). Data were collected in the 14 farms where a comparison between CA and CvtA was established. CA=conservation agriculture, CvtA=conventional system.

Source	SOC Elemental Analyzer		Bulk Density		SOC Stock		TOC Springer- Klee		C-microbial biomass		Basal Respiration		Cumulative Respiration		Biological Fertility Index		QBS-ar		Earthworms		
	Num. df	F	Sig.	F	Sig.	F	Sig.	F	Sig.	F	Sig.	F	Sig.	F	Sig.	F	Sig.	F	Sig.	F	Sig.
Farm(Implementation_Year)	11	36.2	0.00	59.8	0.00	16.0	0.00	27.4	0.00	17.1	0.00	10.6	0.00	18.18	0.00	8.7	0.00	3.8	0.00	11.4	0.00
Implementation_Year	2	2.5	0.09	97.4	0.00	6.7	0.00	3.8	0.03	4.2	0.02	8.9	0.00	7.48	0.00	1.4	0.26	19.4	0.00	32.7	0.00
Agricultural_Systems	1	20.3	0.00	1.4	0.25	9.3	0.00	97.8	0.00	74.7	0.00	4.5	0.04	23.78	0.00	43.5	0.00	23.0	0.00	62.2	0.00
Agricultural_Systems x Implementation_Year	2	6.0	0.00	8.2	0.00	2.7	0.07	7.6	0.00	34.5	0.00	1.6	0.21	11.35	0.00	19.6	0.00	3.8	0.03	9.9	0.00
Agricultural_Systems xFarm	11	4.0	0.00	4.5	0.00	2.8	0.01	9.0	0.00	5.8	0.00	1.8	0.08	3.46	0.00	5.1	0.00	2.7	0.01	5.2	0.00

Table 5. Means of the carbon-related variables, bulk density, biological fertility index, QBS-a, and earthworms count. Means followed by different letters significantly differ (Sidak post-hoc test) between agricultural systems within implementation years. Data were collected in the 14 farms where a comparison between CA and CvtA was established. CA=conservation agriculture, CvtA=conventional system., Long-term=before 2006, Medium-term=from 2006 to 2013, Short-term=after 2013. N.=number of cases, S.D.=Standard deviation.

								A	gricultura	l_systems:	:									
									CA vs.	CvtA										
Implementation Year	SOC Ele Ana	emental lyzer	Bulk Density		SOC Stock		TOC Springer- Klee		C-microbial biomass		Basal Respiration		Cumulative Respiration		Biological Fertility Index		QBS-ar		Earthworms	
	[g k	.g <sup>-1</sup> ]	[Mg m <sup>-3</sup> ]		[Mg ha <sup>-1</sup> ]		[g kg <sup>-1</sup> ]		[µg g <sup>-1</sup>	[µg g <sup>-1</sup> dry soil]		$[mg CO_2-C h^{-1} kg^{-1}$ soil]		C h <sup>-1</sup> kg <sup>-1</sup> vil]	[-]		[-]		[-]	
Long-term	15.3	14.7	1.41	1.40			17.1	15.5	192.0	174.8			310	277	17.8	16.7	69.9	61.2	18.5a	4.7b
	N. 18	N. 18	N. 18	N. 18			N. 18	N. 18	N. 18	N. 18			N. 18	N. 18	N. 18	N. 18	N. 18	N. 18	N. 18	N. 18
	S.D. 0.4	S.D. 0.5	S.D. 0.2	S.D. 0.2			S.D. 4.3	S.D. 4.3	S.D. 91	S.D. 116.3	3		S.D. 169.1	S.D. 143	S.D. 3.2	S.D. 2.4	S.D. 24.4	S.D. 15.7	S.D. 11.1	S.D. 4.7
Medium-term	16.6a	14.6b	1.26b	1.33a	61.9a	60.1b	16.1a	12.9b	203.9a	99.9b	6.7a	5.5b	345a	296b	19a	16.4b	90.6a	66.9b	14.8a	8.2b
	N. 36	N. 36	N. 36	N. 36	N. 90	N. 84	N. 36	N. 36	N. 36	N. 36	N. 90	N. 84	N. 36	N. 36	N. 36	N. 36	N. 36	N. 36	N. 36	N. 36
	S.D. 0.4	S.D. 0.3	S.D. 0.2	S.D. 0.2	S.D. 13	S.D. 12	S.D. 4.9	S.D. 2.8	S.D. 139.	4 S.D. 51.7	S.D. 6.2	S.D. 4	S.D. 161.2	S.D. 138.4	S.D. 3.1	S.D. 3	S.D. 25.6	S.D. 26.6	S.D. 11.6	S.D. 9.3
Short-term	14.7	14.2	1.45	1.43			15.9	14.6	134.8	176.0			258	266	17.3	17.7	63.1	54.5	5.2	1.9
	N. 36	N. 30	N. 36	N. 30			N. 36	N. 30	N. 36	N. 30			N. 36	N. 30	N. 36	N. 30	N. 36	N. 30	N. 36	N. 30
	S.D. 0.3	S.D. 0.2	S.D. 0.1	S.D. 0.1			S.D. 2.5	S.D. 2.1	S.D. 65.2	2 S.D. 114.2	2		S.D. 85	S.D. 86.5	S.D. 2.1	S.D. 2.4	S.D. 23.4	S.D. 20.1	S.D. 7.4	S.D. 3.6

Table 6. Results of the linear mixed models applied to yield data. Agricultural\_Systems (CA vs. CvtA), Implementation\_Year (Long-term=before 2006, Medium-term=from 2006 to 2013, Short-term=after 2013), farm nested within the Implementation\_Year. CA=NT, except for Cerutti and Don Bosco where it was MT, CvtA=plowing, except for Carpaneta, Grandi, where it was MT. Means followed by different letters significantly differ (Sidak post-hoc test) between agricultural systems and implementation years, separately. CA=conservation agriculture, CvtA=conventional system. N.=number of cases, S.D.=Standard deviation.

			Maize			Sovbean		V	Vinter Whea	t
			1. Iuille			20,000m		Num.		
		Num. Df	Den. Df	Sig.	Num. Df	Den. Df	Sig.	Df	Den. Df	Sig.
Farm(Implementation_Year)		10	33	0.11	7	29	0.10	5	14	0.02
Implementation_Year		2	33	0.10	2	29	0.05	2	14	0.03
Agricultural_System		1	33	0.07	1	29	0.01	1	14	0.51
Agricultural_System x Implementation_Year		2	33	0.96	2	29	0.88	2	14	0.69
Agricultural_System x Farm		9	33	0.54	5	29	0.67	4	14	0.61
				Mean			Mean			Mean
		N.	S.D.	[Mg ha <sup>-1</sup> ]	N.	S.D.	[Mg ha <sup>-1</sup> ]	N.	S.D.	[Mg ha <sup>-1</sup> ]
Agricultural_system:	CvtA	30	2.67	9.49	22	0.81	3.37 a	14	1.29	4.85
	CA	29	3.37	7.74	25	0.74	2.58 b	15	1.32	4.26
Group of Implementation_Year:	Long-term	14	3.01	10.2	16	0.76	2.99	4	0.53	4.5 b
	Medium-term	30	3.45	7.93	25	0.91	3.09	21	1.26	4.27 b
	Short-term	15	1.86	8.62	6	0.63	2.25	4	1.44	6.0 a

Table 7. Results of the simplified economic balance that was calculated to estimate (i) the costs associated to the raw material purchase (i.e. herbicides, crops seeds, mineral fertilizers, fungicides) and field operations (i.e. tillage, weeding, harvest, haying, slurry application), (ii) gross gain and the economic efficiency ratio (i.e. revenue from grain yield over direct costs). B=barley, M=maize, R=rice, S=soybean, W=winter wheat.

											Raw m	aterial pu	ırchase	0]	peration	5	G	ross Gai	n	Economic Efficiency			
	Grain Yield [Mg ha <sup>-1</sup> ]											[€ ha-1]			[€ ha-1]			[€ ha-1]		[€ ha-1/€ ha-1]			
	2014- 2013-2014 2015 2015-2016					2013- 2014	2014- 2015	2015- 2016															
Carpaneta		2010	201	<u>.</u>		010		2010	201	•	2014	2010	2010	2014	2010	2010	2014	2010	2010	2011	2010	2010	
NT	S	3.6			М	9.2	W	7.2			562	638	264	125	94	42	861	952	1027	2.3	2.3	4.4	
MT	S	3.7			М	10.2	W	6.9			375	429	269	78	117	117	1138	1321	891	3.5	3.4	3.3	
La Fattoria																							
NT	М	13			S	3.5	В	5.3	S	2.3	655	389	408	203	153	221	1567	963	1282	2.8	2.8	3.0	
CvtA	М	14			S	3	В	5.4	S	3.2	822	367	408	303	212	319	1500	711	1589	2.3	2.2	3.2	
Mosca																							
NT	R	5.4			R	4.9	R	5.2			185	185	142	108	111	104	1597	1419	1573	6.5	5.8	7.4	
CvtA	R	6.2			R	6.3	R	5.9			185	185	142	164	161	158	1821	1859	1765	6.2	6.4	6.9	
Pasti																							
NT	В	4.4	S	1.9	М	10.1	S	3.8			593	426	410	127	105	109	863	1317	1115	2.2	3.5	3.1	
CvtA	В	3.2	S	3.2	Μ	11.1	S	3.8			503	397	238	125	145	123	1305	1490	1274	3.1	3.7	4.5	
Ruozzi																							
NT	W	6.5			М	6.7	W	7.3			254	464	162	321	532	139	627	230	1050	2.1	1.2	4.5	
CvtA	W	7.5			Μ	8.8	W	6.9			254	442	114	612	549	126	521	619	1037	1.6	1.6	5.3	

Figure 1. Location in the Po Valley (Northern Italy) of the farms involved in the study.



Figure 2a, 2b, 2c, 2d, 2e, 2f. Soil organic carbon (SOC) relative content stock, microbial biomass carbon, biological fertility index, QBS-ar, and number of earthworms observed in the topsoil (0-0.3 m) under the different combinations of tillage practices (CvtA, MT, NT) and Implementation\_Year (Long-term=before 2006, Medium-term=from 2006 to 2013, Short-term=after 2013). Cross indicates the mean for each displayed combination. CvtA=conventional system, MT=minimum tillage, NT=no-till. CA=conservation agriculture, CvtA=conventional system. N.=number of cases, MSE=.mean standard error.



**Implementation Year** 

Figure 3. Relative effect of CA on SOC content and stock with respect to CvtA, expressed as  $(SOC_{CvtA} - SOC_{CA})/SOC_{CvtA}$  and  $(STOCK_{CvtA} - STOCK_{CA})/STOCK_{CvtA}$ , which was calculated on 2016 data for each farm where a comparison was established. Data were then averaged by each Implementation\_Year group. Bars are the confidence interval at 95%, which were calculated using the Z scores given the homogeneity of variance and the normal distribution. CA=conservation agriculture, CvtA=conventional system.



Figure 4. Exponential regression between the soil organic carbon content (TOC, measured via the Springer-Klee method) and the microbial cumulative respiration (samples collected in 2016 in topsoil, 0-0.3 m). The number of pairs is 131.



Figure 5. Number of cases of each Biological Fertility Index class in 2014 and 2016 for each tillage system (CvtA, MT, NT) and Implementation\_Year group (long-term=before 2006, medium-term=from 2006 to 2013, short-term=after 2013). CvtA=conventional system, MT=minimum tillage, NT=no-till.



Classes Biological fertility Index  $(0 \rightarrow 30)$ 

Figure 6. Grain yield of maize, winter wheat and soybean under CA (=NT, except for Cerutti and Don Bosco where it was MT) compared to CvtA (=plowing, except for Carpaneta, Grandi, where it was MT). Data are split by Implementation\_Year (long-term=before 2006, medium-term=from 2006 to 2013, short-term=after 2013). Cross indicates the mean for each displayed combination. N=number of cases, MSE=mean standard error.



Long-term Medium-term Short-term Implementation Year

Figure 7. Tradeoff index: ratio of the variation in SOC stock over the variation in C-yield passing from CvtA to NT (from MT to NT in Arisi, Carpaneta, Grandi). CvtA=conventional system, MT=minimum tillage, NT=no-till.

