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**CHALLENGES OF CONSERVATION AGRICULTURE ON SILTY SOILS.
DISENTANGLING THE EFFECTS OF CONSERVATION PRACTICES ON
SOIL ORGANIC CARBON CYCLE AND SOIL PORE NETWORK IN NORTH-
EASTERN ITALY**

Direttore della Scuola: Ch.mo Prof. Antonio Berti

Supervisore: Ch.mo Prof. Francesco Morari

Dottoranda: Ilaria Piccoli

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Ilaria Piccoli, Legnaro 15/01/2017



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Riassunto

La perdita di sostanza organica è una delle minacce del suolo riconosciute a livello europeo e le ripetute lavorazioni del terreno sono state connesse con alcuni effetti negativi sulle proprietà del suolo e con i relativi servizi ecosistemici. Per questo, lo studio di pratiche agronomiche più sostenibili rappresenta una sfida per l'intera comunità scientifica. Tra le tecniche agronomiche sostenibili, l'agricoltura conservativa (AC) è una pratica ampiamente diffusa che è basata su tre principi cardine: 1) minimo disturbo del suolo, 2) copertura permanente del terreno e 3) diversificazione delle colture. AC è spesso associata a numerose funzioni del suolo quali l'aumento della biodiversità, dello stock di carbonio organico e della stabilità degli aggregati e la riduzione del runoff, dell'erosione, delle lisciviazioni di P e delle emissioni di anidride carbonica. Nonostante ciò, recentemente AC non è sempre considerata come una soluzione vincente per la mitigazione del clima e per il miglioramento dell'agroecosistema in quanto l'assenza delle lavorazioni del terreno possono influenzare negativamente lo sviluppo radicale mediante un aumento della densità e della resistenza del suolo e mediante una diminuzione della porosità e degli scambi gassosi. Per di più, i benefici delle pratiche conservative sono riconosciuti essere strettamente legati al tipo di clima e suolo. In quest'ottica di risultati contrastanti, maggiori studi sono necessari per studiare e ottimizzare le potenzialità di pratiche agronomiche più sostenibili. Per questi motivi, in questa tesi, è stata condotta una prova di campo comprendente quattro aziende agricole della bassa pianura Veneta caratterizzate da suoli limosi nei quali le pratiche conservative (non lavorazione, cover-crop e ritenzione dei residui) sono state adottate e confrontate con quelle tradizionali.

Il primo obiettivo di questa tesi è stato quello di valutare gli effetti di AC sul ciclo del C. In particolare è stata valutata l'evoluzione del carbonio organico del suolo (COS) sia in termini quantitativi che qualitativi durante un periodo di transizione di tre anni. Lo stock di COS è stato quantificato mediante l'applicazione della massa equivalente fino a 50 cm di profondità mentre l'effetto delle diverse componenti del trattamento conservativo è stato studiato considerando le biomasse delle colture, delle cover-crop e degli apparati radicali e il tipo di lavorazione come fattori separati. La qualità del COS è stata invece caratterizzata analizzando il carbonio umico, le sue frazioni in peso e la biomassa

microbica. Questo studio ha mostrato come dopo un breve periodo di applicazione di tali pratiche, lo stock di COS nel suolo non sia aumentato mostrando piuttosto una diversa ripartizione lungo il profilo. La qualità del carbonio organico ha invece beneficiato delle pratiche conservative con la produzione di sostanze umiche più policondensate.

Il secondo obiettivo ha riguardato lo studio dell'influenza di AC sugli scambi gassosi del suolo mediante l'analisi della permeabilità all'aria, della diffusione, della air-filled porosity e mediante la derivazione di indici di struttura su 144 campioni indisturbati di suolo di 100 cm³. Le analisi hanno evidenziato le scarse proprietà di trasmissione dei suoli limosi indipendentemente dalla pratica agronomica adottata che hanno portato al raggiungimento di valori critici sia per l'aerazione del terreno che per le attività microbiche aerobiche.

Il terzo obiettivo si è focalizzato sulla caratterizzazione dell'evoluzione della struttura del suolo dopo cinque anni dall'adozione delle pratiche di AC. La porosità del suolo è stata analizzata sia mediante l'utilizzo di microtomografie a raggi-x che di porosimetrie a intrusione di mercurio. La porosità totale, la distribuzione dei pori (dalla macro- alla micro-scala) e l'architettura dei pori sono state quantificate su 96 campioni indisturbati raccolti nelle quattro aziende sperimentali. I risultati hanno mostrato come i suoli limosi del Veneto siano "microstrutturati" in quanto la maggior parte della porosità ricade nel range 0.0074-30 µm e come le pratiche conservative abbiano positivamente influenzato la ultramicroporosità (0.1-5 µm) che è strettamente legata alla protezione della sostanza organica.

Concludendo, come evidenziato dallo scarso effetto sul sequestro del C, sugli scambi gassosi e sulla struttura del terreno, i suoli limosi della bassa pianura Veneta hanno mostrato una lenta reazione alle pratiche conservative. Lo scarso contenuto di COS non complessato disponibile all'interazione con le particelle fini del terreno ha ostacolato la formazione di una struttura stabile portando al compattamento del suolo. Nonostante ciò, le pratiche conservative hanno però positivamente influenzato la qualità del C e la ultramicroporosità suggerendo che un ciclo virtuoso tra sostanza organica e struttura del suolo è stato inizializzato. Un periodo di transizione di più lunga durata sembra essere indispensabile per il raggiungimento di un nuovo equilibrio in sistemi conservativi e più studi sui meccanismi che regolano la struttura in suoli limosi risultano inoltre necessari.

Summary

Soil organic matter loss is a widely recognized European soil threat and intensive and repeated tillage operations are known to negatively affect numerous soil properties and ecosystem services. In this view, the study on more sustainable agronomic managements is a pressing need for research community. Between sustainable techniques, conservation agriculture (CA) is nowadays a spread technique based on three main pivotal points: 1) minimum soil disturbance, 2) permanent soil covering and 3) crop diversification. CA is often associated with numerous soil functions such as increasing of soil biodiversity, organic matter stocks and aggregate stability and decreasing of runoff, erosion and P losses and dioxide carbon emissions. Despite the first estimates, CA practices are recently not recognized as a win-win solution for climate mitigation and agro-ecosystem improvement because the absence of tillage operations may impact the crop root growth through an increase in soil strength and bulk density, and reduce soil porosity and gas exchanges and lastly, the overall benefits of CA have been strictly related to soil type and climate. Within this viewpoint of not consistent results, more research is needed to understand and optimize the potential of sustainable agronomic practices. For these reasons, in this work, a field experiment was conducted including four farms on the low-lying plain of Veneto Region characterized by silty soils, in which conservation agriculture practices (no-tillage, cover crops and residues retention) were applied and compared to conventional tillage system.

The first objective of the thesis was to evaluate the effects of CA practices on C cycle. The soil organic carbon (SOC) evolution in terms of both stock quantity and quality was monitored over a 3-yr transition period. The SOC stock was quantified through an equivalent soil mass approach up to 50 cm depth while the influence of each CA component was disentangled considering crop, cover crop and root biomasses, and tillage type as separate factors. The SOC quality was evaluated through humic carbon, its molecular weight distribution and microbial biomass analyses. The study showed that after short period, CA adoption did not increase C stock but rather its distribution within the soil profile while a positive effect was observed on humic carbon with the production of more polycondensed humic substances.

The second objective regarded the evaluation of the soil gas exchange properties in the poorly structured silty soils of the low-lying plain. The effect of conservation agriculture practices on soil pore and gas transport characteristics was studied through the analyses of air permeability, gas diffusivity and air-filled porosity, and the derivation of soil structure indices on 144 undisturbed 100 cm³ soil cores. Gas transport measurements highlighted low transmission properties of the silty soils independently from agronomic management leading to critical value for both soil aeration and microbial aerobic activity.

The third objective focused on the characterisation of the soil structure evolution after 5-yr of conservation agriculture management adoption. The soil pore network was quantified coupling mercury intrusion porosimetry and x-ray micro-tomography to study the total porosity and size distribution, from the macro- to the ultramicro- scale, and its architecture, on 96 undisturbed soil samples collected in the field experiment. Results suggested that silty soils of Veneto plain are micro-structured since much of the porosity is in the 0.0074-30 µm range and CA practices showed a positive influence on the ultramicroporosity range (0.1-5 µm) which are strictly linked to SOC stabilization.

Concluding, silty soils of the Veneto region low-lying plain showed a slow reaction to conservation agriculture practices, as demonstrated by the poor effect on C sequestration, gas-transport characteristics and soil structure improvements. The limited amount of non-complexed organic carbon available for interaction with the soil fines prevented the formation of a more resilient soil structure leading to soil compaction that negated the exploitation of CA-related benefits. Despite such mechanisms, CA practices positively influenced C quality and ultramicroporosity range suggesting that a virtuous cycle between SOC and soil structure has been initiated. Longer transition period will be needed to reach a favourable equilibrium in the CA systems and more studies elucidating the mechanisms of structure improving conditions for silty soils, such as those examined in this study, are also required.

Chapter I

General introduction

Soil organic matter loss and conservation agriculture

Soil organic matter (SOM) decline has been recognised as one of the eight soil threats by the European Commission (COM (2006) 232) due to its pivotal role on both soil fertility and climate mitigation. During the last 50 years, a $1.1 \text{ t ha}^{-1} \text{ y}^{-1}$ depletion rate of soil organic carbon (SOC) was recorded also in Veneto region as a combination of simplified crop systems (e.g. maize monoculture), frequent tillage operations and lack of organic input (e.g. farmyard manure), worsening the soil quality and increasing greenhouse gasses (GHGs) emissions (Morari *et al.*, 2006). The soils of Veneto region are mainly formed by Calcisols and Cambisols (WRB, 2006), characterized by silty texture, poor structural stability and low SOC content (1.5% on average) (Dal Ferro *et al.*, 2016) (Fig.1) and they have traditionally been intensively tilled to provide a suitable seedbed for crop growth.

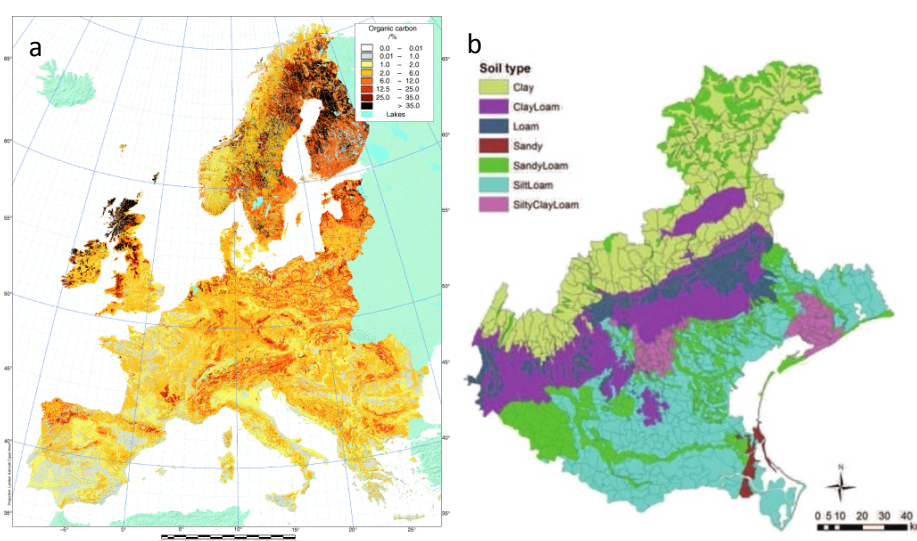


Figure 1- European soil organic carbon content in 0-30 cm soil profile (Jones *et al.*, 2005) (1-a, left). Soils of Veneto region (Dal Ferro *et al.*, 2016) (1-b, right).

Intensive and repeated tillage operations, disrupting soil macro-aggregates and exposing physically-protected intra-aggregate SOC to microbial attack, are general known to negatively affect numerous soil structure properties such as SOC stock, SOC quality and aggregate stability (Balesdent *et al.*, 2000; Devine *et al.*, 2014; Kravchenko *et al.*, 2012;

Six *et al.*, 1998, 1999) that are linked to soil fragmentation, fertility impoverishment, erosion and CO₂ emissions.

Nowadays, the idea that agriculture should not only be high yielding, but also sustainable (Reynolds & Borlaug, 2006) has spread among the scientific community, and conservation agriculture (CA) has been suggested as a widely adapted set of management principles that can assure more sustainable agricultural production (Branca *et al.*, 2011; Lal, 2004; Verhulst *et al.*, 2010). CA was proposed based on three main pivotal points: 1) minimum soil disturbance, 2) permanent soil covering and 3) crop diversification (Vaneph & Benites, 2001) and, as in other European countries, its application in Veneto is also increasing and was subsidised during the two last rural development programmes of the Veneto Authorities (Regione Veneto, 2013, 2016) to reduce production costs, on the one hand, and allegedly to regulate and support several ecosystem services, on the other (Bash, 2005; Kassam *et al.*, 2015). Minimum soil disturbance and, in particular, a no-tillage system, as a result of the absence of soil fragmentation, is related to several soil improvements (Soane *et al.*, 2012) such as an increased aggregate stability (Six *et al.*, 2002) by means of both greater stock (West & Post, 2002) and higher fraction of stable SOC (Bayer *et al.*, 2003; McCallister & Chien, 2000) that can assure a higher soil C sequestration (Lal & Kimble, 1997) and a reduction in erosion risk (Li *et al.*, 2007). No-tillage also positively affects the habitat and activity of soil flora and fauna (Soane *et al.*, 2012) and larger earthworm populations promote the formation of a vertical oriented bio-macropore network that is essential for soil water drainage and aeration improvements (Blackwell *et al.*, 1990a; Horn, 2004). Besides the benefits of no-tillage on soil properties, it is also associated with climate regulation, since it reduces direct CO₂ emissions through less use of agricultural vehicles (i.e. fuel saving) (Smith, 2008; Smith *et al.*, 1998; Soane *et al.*, 2012; West & Post, 2002). A permanent soil covering is, instead, usually achieved by means of both crop residues retention on soil surface and cover-crops (Vaneph & Benites, 2001) and is fundamental for enriching topsoil organic matter, increasing soil bearing capacity, improving infiltration that lead to erosion protection, decreasing leaching and fostering biodiversity (Thierfelder & Wall, 2009; Verhulst *et al.*, 2010). Moreover, by fixing atmospheric nitrogen in the soil, the use of legume cover crops could improve soil fertility (Farooq &

Siddique, 2015). Lastly crop diversification, through different crop rotations, plays a key role in determining the success of CA because this allows the limiting of insects, pests and crop diseases (Witmer *et al.*, 2003).

Despite the first estimates of Smith *et al.* (1998), suggesting that all fossil fuel C emissions from European agriculture could be mitigated through the complete conversion to no-tillage systems, CA practices are nowadays not recognized as a win-win solution for climate mitigation and agro-ecosystem improvement (Powlson *et al.*, 2014; VandenBygaart, 2016a). Firstly, the main difference between conventional and conservation agriculture on SOC stock seems to be just a matter of a different distribution through the soil profile and not of total C stocks (Powlson *et al.*, 2011) and, also as a consequence of too shallow soil samplings (Baker *et al.*, 2007), the effects of CA on climate change mitigation might have been overestimated in the past (VandenBygaart, 2016b). Secondly, the absence of tillage operations has also been recognized to have a negative impact on soil bulk density (Dal Ferro *et al.*, 2014; Palm *et al.*, 2014a), strength (Schjønning & Rasmussen, 2000) and structure (Munkholm *et al.*, 2013) with a significant reduction in water infiltration and storage capacity (Lipiec *et al.*, 2006). Other Authors (Martínez *et al.*, 2016; Mentges *et al.*, 2016) recently also observed a decrease in gas transport-related characteristics (air-filled porosity, air permeability and gas diffusion) as a consequence of soil compaction, which is known to persist for several years after its occurrence (Berisso *et al.*, 2012). Denser and stronger soil characteristics could similarly represent adverse conditions for deep root growth (Baker *et al.*, 2007), which is essential for both good crop establishment and stable soil organic pool (Rasse *et al.*, 2006). Lastly, the overall benefits of CA have been strictly related to soil type and climate. Indeed Soane *et al.* (2012) reviewed the western and south-western European adoption of no-tillage confirming that soils with low structural stability and poor drainage are generally not suitable for no-tillage systems and can lead to a substantial reduction in crop yield. Unstable soils, especially with low organic matter content, are indeed subjected to higher risk of compaction (Ehlers and Claupein, 1994; Van Ouwerkerk and Perdok, 1994) which could be the limiting factor for successful adoption of CA practices.

Thesis objectives and outline

Within this viewpoint of not consistent results, more research is needed to understand and optimize the potential of sustainable agronomic practices (Eden et al., 2012; Farooq and Siddique, 2015; Nakajima and Lal, 2014; Thorbjørn et al., 2008) especially concerning the application of conservation agriculture systems in order to advance the tools used to pursue mitigation strategies. Moreover, the number of European experiments over a wide range of soils, fertilizer applications and climate conditions with crops grown within rotations is still limited and requires expanding (Soane et al., 2012).

For these reasons, in this work, a field experiment was conducted including four farms on the low-lying plain of Veneto Region characterized by silty soils, in which conservation agriculture practices (no-tillage, cover crops and residues retention) were applied and compared to conventional tillage system.

Silty soils of the low-lying plain are considered unstable, with low organic matter content and limited C protection capacity, poor aggregate stability and high risk of compaction.

The first objective of the thesis was to evaluate the effects of CA practices on the SOC cycle and their potential effects on C sequestration. The starting research hypothesis was that in the short term, CA practices increase the vertical stratification of SOC but not the total stock through the soil profile. The soil organic carbon (SOC) evolution in terms of both stock quantity and quality was monitored over a 3-yr transition period. The SOC stock was quantified through an equivalent soil mass approach up to 50 cm depth while the influence of each CA component was disentangled considering crop, cover crop and root biomasses, and tillage type as separate factors. The SOC quality was evaluated through humic carbon, its molecular weight distribution and microbial biomass analyses.

The second objective regarded the evaluation of the soil gas exchange properties in the poorly structured silty soils of the low-lying plain. Soil gas exchange is one of the most important soil functions that directly impacts on crop productivity and the environment. The starting research hypothesis was that CA practices, providing a better soil structure, increase gas transport exchange conditions. The effect of conservation agriculture practices on soil pore and gas transport characteristics was studied through the analyses of air permeability, gas diffusivity and air-filled porosity, and the derivation of soil structure indices on 144 undisturbed 100 cm³ soil cores.

The third objective focused on the characterisation of the soil structure evolution after 5-yr of conservation agriculture management adoption. The starting research hypothesis was that CA practices provide a better soil structure, increasing the macroporosity fraction and its connectivity. The soil pore network was quantified coupling mercury intrusion porosimetry and x-ray micro-tomography to study the total porosity and size distribution, from the macro- to the ultramicro- scale, and its architecture, on 96 undisturbed soil samples collected in the field experiment.

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Chapter II

Disentangling the effects of conservation agriculture practices on the vertical distribution of soil organic carbon. Evidence of poor carbon sequestration in North- Eastern Italy*

*Piccoli I., Chiarini F., Carletti P., Furlan L., Lazzaro B., Nardi S., Berti A., Sartori L., Dalconi M.C., Morari F., 2016. Disentangling the effects of conservation agriculture practices on the vertical distribution of soil organic carbon. Evidence of poor carbon sequestration in North- Eastern Italy. *Agric. Ecosyst. Environ.* 230, 68–78. doi:10.1016/j.agee.2016.05.035

1 Introduction

Conservation agriculture (CA) is a system of agronomic practices that minimizes mechanical soil disturbance (e.g. no-tillage, NT), maintains permanent soil cover by using crop residues and cover crops, and rotates crops.

CA regulates CO₂ emissions by increasing the C stored in soil (i.e. C sequestration) (Lal and Stewart, 2010) and reducing direct emissions through less use of agricultural vehicles (i.e. fuel saving) (Soane et al., 2012). It has been evaluated that CA can enhance soil C stocks by about $0.57 \pm 0.14 \text{ t C ha}^{-1} \text{ year}^{-1}$ in the top 30 cm (West and Post, 2002). Despite the first estimates of Smith et al. (1998), suggesting that all fossil fuel C emissions from European agriculture could be mitigated through the complete conversion to NT, CA is still not recognized as a win-win option for soil C sequestration (Powlson et al., 2011; VandenBygaart, 2016). Ogle et al. (2012) argued that NT could increase or decrease the SOC content depending on its effects (positive or negative) on crop biomass and consequent C input. The unpredictable behaviour of NT on SOC was viewed by the authors as strongly dependent on climatic conditions, which affect plant growth and soil processes and therefore play a key role in organic matter dynamics. Angers and Erik-Hamel (2008) suggested that crop residues left on the soil surface are less persistent than those incorporated by ploughing. Indeed, incorporation promotes the interaction between crop residues and soil particles and in turn enhances the physical mechanisms of SOC protection (Balesdent et al., 2000).

Baker et al. (2007) postulated that the greater C content in NT fields may be an artefact of shallow sampling and that, after considering deeper soil profiles, NT would not show any advantages in C sequestration with respect to conventional tillage. They also suggested that sampling deeper than 30 cm would be required to fully clarify the role of CA on soil C stocks. The meta-analysis by Luo et al. (2010) pointed out that NT could enhance C stocks in the top 10 cm of soil, decrease them in the deeper 10-40 cm layer and be ineffective below 40 cm.

The vertical SOC distribution in CA would be affected not only by non-inversion tillage, but also by root growth and patterns. By modifying the soil structure within the profile, NT would create a structure stratification that negatively affects root-growth and root-induced parameters (e.g. C distribution). Powlson et al. (2011) concluded that the main

difference between conventional and conservation agriculture is just a matter of SOC distribution in the soil profile and not of total C stocks, and as a consequence the effects of CA on climate change mitigation might have been overestimated in the past.

Soil tillage managements were recognized to affect not only the soil C stocks but also their quality (Devine et al., 2014; Six et al., 1998). Soil organic matter (SOM) in conventionally tilled soils was usually associated to a reduction in fulvic acids, humin and labile humus substances (Kravchenko et al., 2012). On the contrary, conservation practices improved SOM quality in the top layers by leading to a higher fulvic acids content (McCallister and Chien, 2000) and lower concentrations of semiquinone free radicals and humification degree of SOM (Bayer et al., 2003).

Soil microbiota is another major driver of organic matter turnover and nutrient cycling (Schloter et al., 2003). The role of the microbial biomass in mediating soil processes and its relatively high turnover rate, logically suggests that the microbial biomass could be a sensitive indicator and early predictor of changing SOM processes in CA (Rincon-Florez et al., 2015).

In spite of its recognized benefits, “European and national administrations are still not fully convinced that the concept of CA is the most promising one to meet the requirements of an environmentally friendly farming” (Basch, 2005; cit. in Friedrich et al., 2014). Very few countries in Europe (e.g. Switzerland, Italy) promote CA with national or regional policies (Friedrich et al., 2014).

In a global change scenario and in order to advance the tools used to pursue mitigation strategies, it is important to quantify the benefits observed during the transition period from conventional to conservation practices and identify the main mechanisms driving SOC dynamics. The aim of this study was to evaluate the SOC evolution over a 3-yrs transition period in three experimental farms on the low-lying plain of Veneto Region. The impact of CA on soil quality was also quantified by monitoring the humic carbon and its molecular weight distribution, and the microbial biomass. In order to improve the monitoring procedures, a massive soil sampling programme was conducted in ca. 150 positions, considering the SOC stratification within a 0-50 cm profile.

2 Materials and methods

2.1 Experimental sites

The experiment was set up on three farms in North-eastern Italy (Fig. 1, Tab. 1). Farm 1 (F1) “Vallevecchia”, is sited on the Adriatic coast ($45^{\circ} 38.350'N$ $12^{\circ} 57.245'E$, -2 m a.s.l.), the soil is Gleyic Fluvisols or Endogleyic Fluvic Cambisols (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 plain ($45^{\circ} 2.908'N$ $11^{\circ} 52.872'E$, 2 m a.s.l.), respectively. Both are characterized by Endogleyic Cambisols (FAO–UNESCO 1990) silty-loam soil, more homogeneous in texture than F1.

The climate is sub-humid, with annual rainfall around 829 mm in F1, 846 mm in F2 and 673 mm in F3. In the median year, rainfall is 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). Reference evapotranspiration (ET_o) is 860, 816 and 848 mm, with a peak in July (4.9, 4.6 and 4.8 mm d⁻¹). ET_o exceeds rainfall from May to September in F1 and F2 and from May to October in F3.



Figure 1 - Experimental sites in the Veneto Region low plain, North-eastern Italy. Farms positions are marked with triangles (F1, F2 and F3).

Table 1 - Main soil physical and chemical characteristics (top 50 cm) of the experimental farms.

Characteristic	Unit	Farm 1	Farm 2	Farm 3
		“Vallevecchia”	“Diana”	“Sasse-Rami”
Sand	g 100 g ⁻¹	34.2	8.3	18.4
Silt	g 100 g ⁻¹	42.6	66.1	57.8
Clay	g 100 g ⁻¹	23.2	25.6	23.8
pH		8.3	8.0	8.6
Carbonate	g 100 g ⁻¹	53.0	4.0	13.0
Active Carbonate	g 100 g ⁻¹	3.0	1.0	3.0
Organic carbon	g 100 g ⁻¹	1.0	0.9	0.8
Assimilable P	mg kg ⁻¹	32.0	22.0	6.0
Exchangeable Ca	meq 100 g ⁻¹	24.7	21.7	15.5
Exchangeable Mg	meq 100 g ⁻¹	3.2	3.4	1.4
Exchangeable K	meq 100 g ⁻¹	0.5	0.3	0.2

2.2 The experiment

The field experiments were established in October 2010 in order to compare conventional (CONV) versus conservation (CONS) management systems. Cultivation protocols in CONS were set up according to the Measure 214 – Sub-Measure i, “Eco-compatible management of agricultural lands” of the Rural Development Programme (RDP) supported by the Veneto Region (Regione Veneto, 2013).

The crop rotation (four-year) was the same in both treatments: wheat (*Triticum aestivum* L.), oilseed rape (*Brassica napus* L.), maize (*Zea mays* L.) and soybean (*Glycine max* (L.) Merr.). In CONS, cover crops were also grown between the main crops: sorghum (*Sorghum vulgare Pers. var. sudanense*) during spring-summer and a mixture of vetch (*Vicia sativa* L.) and barley (*Hordeum vulgare* L.) in autumn-winter. Conversely, the soil remained bare between the main crops in CONV.

Rotation was in contemporary phases for a total of 24 fields (2 treatments x 4 crops x 3 farms), with the CONS and CONV treatments adjacent. Experimental fields were rectangular (about 400 m length x 30 m width) with an average size of 1.2 ha.

CONV operations included a 35-cm depth ploughing (by multiboard plough) with crop residues incorporation and seedbed preparation by disk arrow (<15 cm depth). CONS management consisted of a no-tillage approach with cover crop suppression, direct sowing, harvesting with crop residues left on soil surface and cover crop sowing.

In both management systems, localized mineral fertilization was applied before the sowing for all crops, integrated with a side dressing treatment in maize and wheat. There was no additional fertilization for cover crops, according to the Sub-Measure protocol.

Pesticide applications depended on crop requirements, assessed by IPM implementation and were the same for both CONV and CONS. Before spring seeding, N-(phosphonomethyl) glycine was applied to suppress the winter cover crop in CONS. Suppression of sorghum in CONS was mechanical, through shredding.

2.3 Crop residues and root biomass

In each field, crops residues were collected after the harvest in three 1 m² sampling areas. To determine the dry weight, the biomass was dried at 65 °C in a forced draft oven for 72 h. The total root biomass in the upper 50 cm layer was determined according to the monolith method (Böhm, 1979) excavating a 0.3 m × 0.3 m × 0.50 m monolith in each sampling area. Once separated from the soil particles by washing, roots were oven-dried at 65 °C to determine the dry weight.

2.4 Soil sampling

Sampling was performed in 2011 (“T0”) and 2014 (“T1”) during the spring (3-yrs interval). Undisturbed soil cores (profile 0-50 cm) were collected in 6 positions per field according to a systematic sampling scheme, using a hydraulic sampler. The same points were sampled in the two campaigns, identifying the positions using a GNSS with Real Time Kinematic (RTK) correction (precision of ca. 2 cm). Soil cores were then cut to extract three layers, 0-5 cm (L1), 5-30 cm (L2), 30-50 cm (L3), and stored at 5 °C prior to physical and chemical analyses. There were additional campaigns in May and

September 2012, 2013 and 2014 to collect disturbed samples in the 0-30 cm profile for microbial biomass analyses.

864 samples were analyzed for bulk density, particle size distribution, organic carbon and total nitrogen, according to the factorial combination of 6 sampling positions \times 3 layers \times 2 treatments \times 4 crops \times 3 farms \times 2 years. Due to the complexity and time required, carbon quality and microbial biomass were determined only on a reduced number of samples, obtained by bulking the single samples collected at 0-30 cm in each field.

Carbon quality was measured for 48 samples, according to the factorial combination of 1 bulked sample per field \times 2 treatments \times 4 crops \times 3 farms \times 2 years (beginning and end of the experiment). 144 samples were analyzed for microbial biomass, 1 bulked sample per field \times 2 treatments \times 4 crops \times 3 farms \times 2 dates (i.e. May and September) \times 3 years (2012, 2013 and 2014).

2.4.1 Soil physical and chemical analyses

Samples were weighed and a fraction was oven-dried at 105 °C for 24 h to calculate bulk density by the core method (Grossman and Reinsch, 2002). The remaining sample fraction was air-dried, and sieved at 0.5 mm for organic C and N determination by the flash combustion method using a CNS Elemental Analyzer (Vario Max, Elementar Americas, Inc., Germany) after removal of inorganic C with acid pretreatment.

For particle size distribution determination, samples were sieved at 2 mm, dispersed in 2% sodium hexametaphosphate solution and shaken for 12 h at 80 rpm. Particle size distribution was determined through laser diffraction method (Mastersizer 2000, Malvern Instruments). A dedicated algorithm was used to convert diffraction values into pipette ones.

A representative soil sample for each farm was analyzed by X-ray powder diffraction (XRPD) in order to determine the mineral composition. Analyses focused on the identification of clay minerals (swelling and not swelling clay minerals) contained in the $< 2 \mu\text{m}$ size fraction (clay fraction). Portions of the bulk soil samples were immersed in demineralized water and dispersed using a laboratory stirrer. The obtained suspensions were centrifuged and the washing water eliminated. Demineralized water was then

added and sediments re-dispersed; the clay fraction ($< 2 \mu\text{m}$) was separated by decantation and withdrawn by a syringe. Oriented specimens were prepared by depositing the clay fraction suspension on aluminium sample holders and drying at room conditions. The oriented specimens were also treated with ethylene glycol vapour (EG) to verify the presence of swelling clay minerals, and one oriented specimen was heated to $350 \text{ }^\circ\text{C}$ to identify the presence of chlorite/vermiculite. Sediments for bulk specimen preparation were ground under water for 5 min using a McCrone micronizing mill. A known amount (20 wt%) of zincite (ZnO) was added to the powder samples as internal standard. The addition of an internal standard is required for quantifying the amorphous/poorly crystalline phases possibly present in bulk samples. X-ray diffraction data were collected using a Panalytical X'Pert PRO MPD diffractometer equipped with a X'Celerator detector, a Co-anode X-ray tube and operating in Bragg-Brentano reflection geometry. Divergence and antiscatter slits of $\frac{1}{4}^\circ$ and $\frac{1}{2}^\circ$ aperture respectively and 0.04 rad Soller slits were used as incident beam optics. The oriented specimens were measured in the 2° - 40° interval counting 30 sec per step, whereas for random powder mounts data were acquired in the 4° - 84° interval counting 100 sec per step. Quantitative estimates of individual minerals were obtained by full profile analyses of diffraction data applying the Rietveld method as implemented in Topas v4.1. Swelling clay minerals typically exhibit high structural and stacking disorder, and an accurate structural model as required by the Rietveld method is not easily implemented. This problem was overcome by considering the swelling clay minerals as amorphous phases. Humic substances (HS) were extracted from 2 mm-sieved, air-dried samples with 0.1 mol L⁻¹ KOH, pH 13.5, (1:10 w/v) at $50 \text{ }^\circ\text{C}$ for 16 h in a N₂ atmosphere, and freed from the suspended material by centrifugation at 7000 g for 20 min and filtration on Whatman 42 filter paper (Whatman, Maidstone, England) (Carletti et al., 2009). Here, the term HS is the fraction soluble in bases and comprehensive of humic and fulvic acids. Humic extract organic carbon contents were assayed by dichromate oxidation (Walkley and Black, 1934). Molecular-weight distribution and gel-permeation chromatography of each humic extract was performed with the method of Dell'Agnola and Ferrari (1971) on a Sephadex G-100

gel packed in a 70 × 1.6 cm Pharmacia column (Pharmacia, Uppsala, Sweden) as described in Cardinali et al. (2014)

The gel packing solution and eluent were both 20 mM Na₂B₄O₇. The apparent molecular sizes of HS were >100 kDa (High Molecular Size, HMS), 100–10 kDa (Medium Molecular Size, MMS) and <10 kDa (Low Molecular Size LMS). The column calibration was based on a standard kit for molecular weights (Sigma-Aldrich Gel Filtration Molecular Weight Markers MWGF200). All determinations were performed in triplicate.

The microbial biomass-C and -N (micr C and micr N) contents were determined by the fumigation-extraction method (Sparling and West, 1988) as reported in Carletti et al. (2009). For each soil sample three fumigated and three non-fumigated aliquots were analyzed. Fumigation was performed under vacuum in a glass desiccator containing 3 ml H₂O, 2 g of NaOH pellets and a beaker filled with glass beads and 50 ml of chloroform. After 16 h of incubation in the dark, the apparatus was degassed thoroughly, and fumigated soil was transferred in a centrifuge tube to which 0.5 M K₂SO₄ was added at a 1:4 w/v ratio. After 30 min of rotatory shaking at 120 rpm the sample was centrifuged for 5 min at 6500 g and the supernatant filtered through a Whatman no. 4 filter and kept in polyethylene tubes at –20 °C until further analyses. As a reference for subtraction, the same K₂SO₄ extraction was performed on a corresponding aliquot of non-fumigated soil. Organic C content in the extracts was determined by dichromate digestion (Kirchner et al., 1993). Nitrogen content was determined according to Cabrera and Beare (1993). The microbial biomass carbon and nitrogen were obtained by subtracting the additional carbon and nitrogen liberated by the fumigation procedure from the directly extractable amounts of organic C and N in the soils, and calculated by applying a conversion coefficient (Brookes et al., 1985; Vance et al., 1987).

2.4.2 Soil organic carbon and total nitrogen stocks

The equivalent soil mass (ESM) method (VandenBygaart and Angers, 2006) was applied in order to normalize the effects of tillage on bulk density (Post et al., 2001) in SOC and TN stock calculation.

According to the minimum ESM (Lee et al., 2009) the equivalent soil organic carbon (SOC_{equiv}) ($t\ ha^{-1}$) stock was calculated as follows:

$$SOC_{equiv} = SOC(0 - a) - \left(\left(\frac{SOC_{dl}}{h_{dl}} \right) \times \left(\frac{(SM(0 - a) - MSM) \times 0.0001}{BD_{dl}} \right) \times 100 \right)$$

where SOC (0-a) is the SOC in the 0-a soil profile ($t\ ha^{-1}$), SOC_{dl} the SOC in the deepest layer ($t\ ha^{-1}$), h_{dl} the deepest layer height (cm), SM(0-a) the soil mass in 0-a soil profile (kg), MSM the minimum soil mass in 0-a soil profile (kg) and BD_{dl} the bulk density of the deepest layer. The same equation was used for TN stock.

The minimum ESM was applied for incremental layers, considering first L1 (0-5 cm, reference soil mass of $398\ t\ ha^{-1}$), then L1+L2 (0-30 cm, reference soil mass of $2\ 384\ t\ ha^{-1}$) and finally the entire soil profile L1+L2+L3 (0-50 cm, reference soil mass of $6\ 368\ t\ ha^{-1}$).

2.5 Statistical analysis

Data were analyzed with a linear mixed-effect model based on REML (Restricted Maximum Likelihood) estimation method, considering clay and sand content as continuous factors and the treatment, layer, year and farm (random factor) as categorical factors. Data of each treatment belonging to the same field were considered as sub-replicated and treated as nested measures.

Since spatial autocorrelation of data residual errors was not significant (Moran's test I), mixed model results were not corrected for spatial autocorrelation (Schabenberger and Pierce, 2001). All possible first and second order interactions between factors were tested, selecting the model with the smallest AIC (Akaike's Information Criterion) (Schabenberger and Pierce, 2001). Post-hoc pairwise comparisons of least-squares means (LSE) were performed, using the Tukey method to adjust for multiple comparison. Statistical analyses were performed with SAS software (SAS Institute Inc. Cary, NC, USA), 5.1 version.

Treatment factor compares only the integrated effects of conservation versus conventional agriculture, therefore an additional mixed model was applied in order to disentangle the individual influences of management practices on SOC stocks. The model considered

the residues and root biomass of crops and cover crops as continuous factors and the tillage type (no-tillage vs conventional tillage) as categorical factor.

3 Results

3.1 Residues and root biomass

Aboveground residues production of crops and cover crops amounted to 25.5 t ha⁻¹ in 3 years with slightly higher values in CONV (26.6 t ha⁻¹) than CONS (24.4 t ha⁻¹), even if not significant (Tab. 2). Soil texture influenced the results ($p < 0.05$), the sand being negatively correlated with the residue biomass. On the contrary, the treatments were discriminated by root biomass, with CONS (6.8 t/ha) > CONV (5.4 t/ha), while no specific interactions were observed with the texture or “farm” factors.

Table 2 - Comparison of significance level among the linear mixed-effect models analysis of residue and root biomasses, bulk density, soil organic carbon (SOC) concentration, total nitrogen (TN) concentration, C/N ratio, humic C, high molecular size humic fraction (HMS), medium molecular size humic fraction (MMS), low molecular size humic fraction (LMS), microbial C (micr C) and microbial N (micr N).

Effect	Residue	Root	Bulk density	SOC concentration	TN concentration	C/N	Humic C	HMS	MMS	LMS	Micr C	Micr N
Intercept	0.08	0.28	<0.01	0.05	0.03	0.02	0.25	0.27	0.03	0.04	0.03	0.04
Treatment	0.40	0.03	<0.01	<0.01	<0.01	0.68	0.05	0.88	0.05	0.06	0.22	0.33
Year	*	*	0.04	0.06	<0.01	<0.01	0.04	0.26	<0.01	<0.01	<0.01	<0.01
Layer	*	*	<0.01	<0.01	<0.01	0.02	#	#	#	#	#	#
Sand (g 100 g ⁻¹)	0.04	0.83	0.36	<0.01	<0.01	0.06	0.26	0.53	0.50	0.11	<0.01	0.02
Clay (g 100 g ⁻¹)	0.26	1.00	<0.01	<0.01	<0.01	0.01	0.95	0.71	0.33	0.20	0.04	0.09
Layer × Treatment	-	-	<0.01	<0.01	<0.01	0.51	-	-	-	-	-	-
Year × Treatment	-	-	0.22	-	0.96	0.55	0.78	0.44	0.11	0.16	-	-
Year × Layer	-	-	<0.01	-	-	-	-	-	-	-	-	-
Farm × Treatment	-	-	-	-	-	-	<0.01	<0.01	0.01	<0.01	-	-
Year × Layer × Treatment	-	-	-	-	<0.01	0.27	-	-	-	-	-	-
Farm × Treatment × Year	-	-	-	-	-	-	0.73	0.25	0.15	0.04	-	-

* Residue and root biomasses were evaluated only in terms of cumulative biomass without testing the year effect in the top 50 cm of soil. - Variables not considered in the linear mixed-effect models according to lowest Akaike's Information Criterion. # Carbon quality and microbial biomass were determined only in the top 30 cm of soil.

3.2 Bulk density

Bulk density (BD) showed significant differences ($p < 0.01$) according to depth, increasing from 1319 kg m^{-3} at 0-5 cm to 1515 kg m^{-3} at 30-50 cm, and treatments, with higher values in CONS (1450 kg m^{-3}) than CONV (1385 kg m^{-3}). Differences between the two management systems were observed only for the first two layers (interaction tillage \times layer significant at 0.01) as shown in Fig. 2. BD also varied according to the texture, being negatively correlated with clay content ($p < 0.01$), but not with sand (Tab. 2). From 2011 to 2014, BD showed a slight but significant ($p = 0.04$) reduction from 1426 kg m^{-3} to 1409 kg m^{-3} in both management systems, since the correlation year \times treatment was not significant ($p = 0.22$) (Tab. 2). A temporal effect was observed only in the top two layers while BD resulted as unaffected in the deeper one (year \times layer, $p < 0.01$).

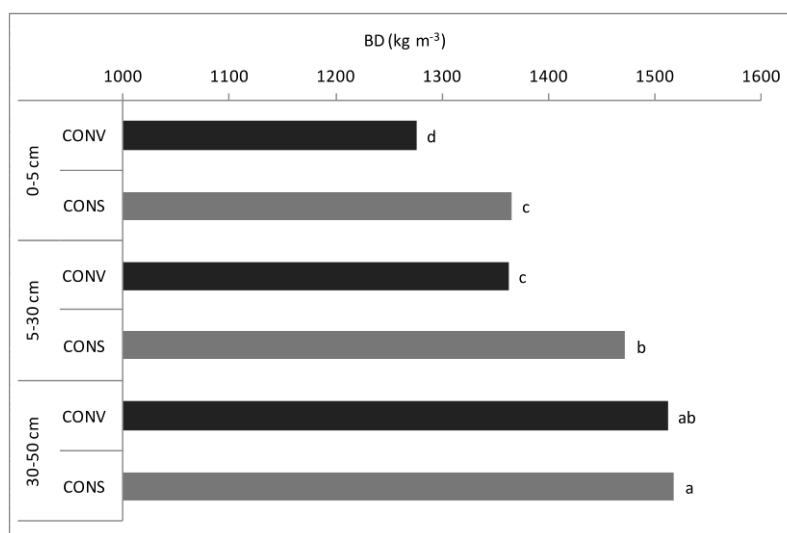


Figure 2 - Bulk density distributions at different soil layers in conventional (CONV) and conservation (CONS) management systems. Bulk density differs significantly when labelled with different letters (Tukey post hoc test with $p \leq 0.05$; $n = 144$).

3.3 Soil organic carbon and total nitrogen concentrations

Soil organic carbon (SOC) concentration showed significant differences ($p < 0.01$, Tab. 2) according to the treatment and depth. SOC was on average 0.99% in CONS and 0.91% in CONV, a result strongly influenced by SOC at 0-5 cm, 1.20% in CONS and 0.98% in CONV (interaction layer \times treatment significant at 0.01) (Fig. 3). Below 5 cm depth, no

differences were observed between treatments (0.91% at 5-30 cm and 0.84% at 30-50 cm).

SOC content was also influenced by the texture (Tab. 2), being positively correlated with clay and negatively with sand. The relationships between SOC and clay in the individual farms are shown in Fig. 4. Clay content represented 59% and 41% of the variability in F1 and F3 respectively, while the correlation was not significant in F2.

Total nitrogen (TN) also discriminated the two treatments, with higher values in CONS (0.143%) than CONV (0.135%) ($p < 0.01$, Tab. 2). As observed for SOC, there were significant differences only in the top layer (treatment \times layer significant at 0.01) (Fig. 3). TN decreased according to the depth, year ($p < 0.01$), from 0.149% in 2011 to 0.129% in 2014, and sand content.

The final C/N ratio resulted as 7.1 without a significant influence of the treatments (Tab. 2). C/N oscillated according to the layer ($p < 0.05$) from 7.3 at 0-5 cm to 6.9 at 5-30 cm and 7.0 at 30-50 cm. In addition, C/N increased over the study period ($p < 0.01$) from 6.5 in 2011 to 7.6 in 2014 and proportionally to the clay content.

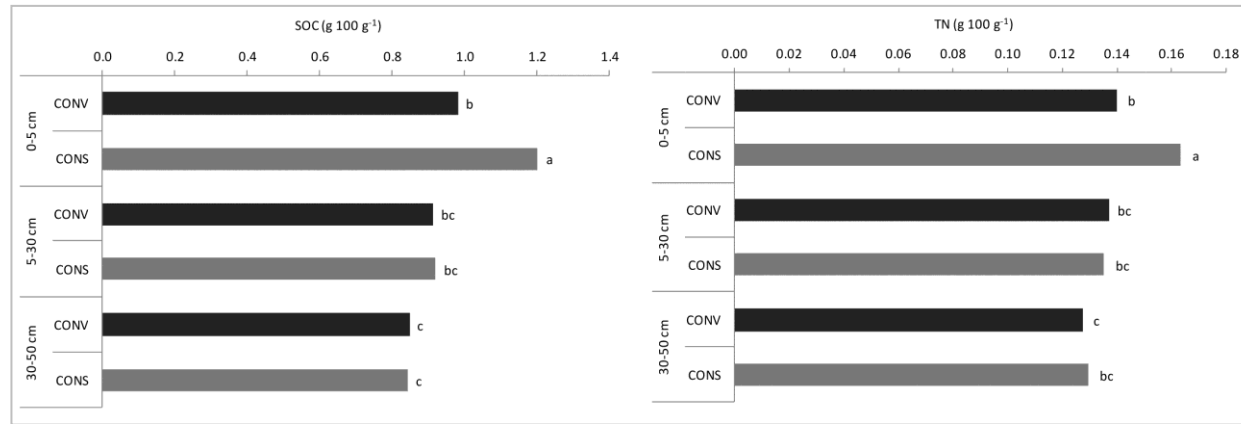


Figure 3 - Soil organic carbon (SOC) (left) and total nitrogen (TN) (right) concentration at different soil layers in conventional (CONV) and conservation (CONS) management systems. SOC and TN differ significantly when labelled with different letters (Tukey post hoc test with $p \leq 0.05$; $n=144$).

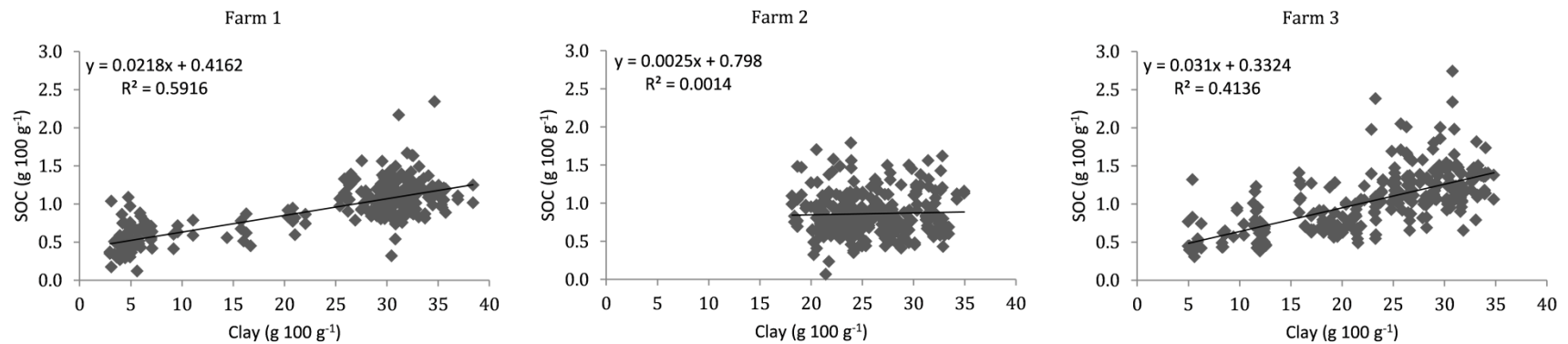


Figure 4 - Relationships between clay and soil organic carbon (SOC) concentrations in the experimental farms.

3.4 Humic carbon and microbial biomass carbon and nitrogen

Soil C in humic extracts (HC) resulted as significantly higher in CONS (1.6 mg HC g⁻¹) than CONV (1.5 mg HC g⁻¹), while a 0.02% decrease was recorded between 2011 and 2014 (Tab. 2).

Humic molecular weight fractions evidenced no differences in high molecular weight (HMS) peak for treatment (Fig. 5) or year, being 9.92% of the HC on average.

Medium molecular size humic fraction (MMS) content resulted as significantly different between CONV and CONS (58.26% and 61.58% respectively) and between 2011 and 2014 (64.16% and 55.68% respectively). Within the farms, treatments influenced the second peak content only in F2 where CONS soils resulted in significantly higher content (farm × treatment interaction significant at p=0.01, Tab. 2).

Finally, the smallest humic fraction (LMS) content showed differences among treatments (significant at p=0.06, Fig. 5) and years. Only F2 evidenced responses to soil management in terms of LMS content, being lower in CONS than CONV (28.38% and 39.93% respectively).

Microbial C and N parameters averaged 170 mg kg⁻¹ and 10 mg kg⁻¹ respectively, resulting in no significant differences either between treatments or among farms. However, for the 0-30 cm profile data there were significant differences according to texture and in the three years, with a lower content of both micr C and N in 2013 when compared with 2012 and 2014 (Fig.6).

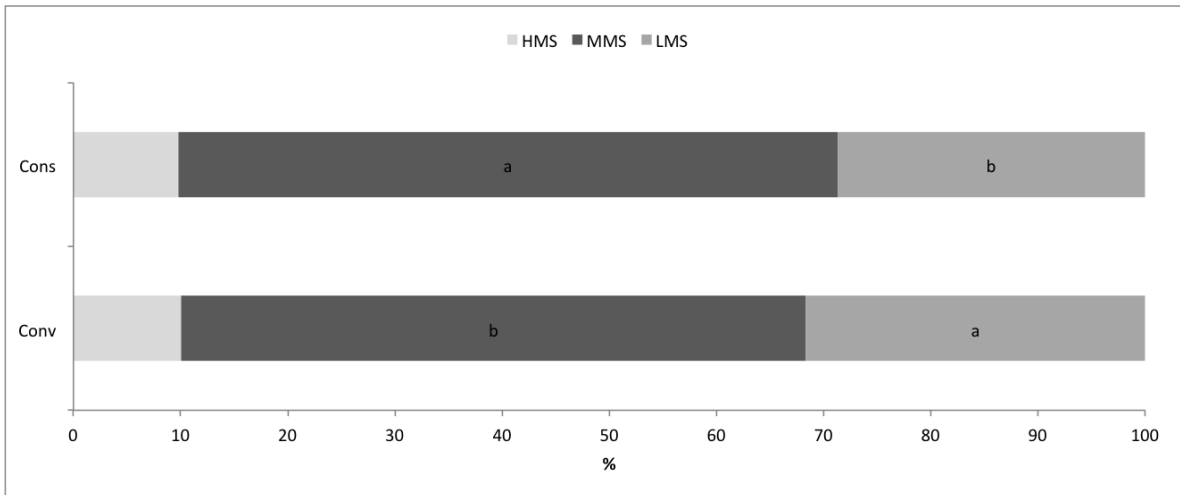


Figure 5 - Humic molecular weight fractions in conventional (CONV) and conservation (CONS) management systems. HMS: high molecular size humic fraction, MMS: medium molecular size humic fraction, LMS: low molecular size humic fraction. Molecular weight fractions differ significantly when labelled with different letters (Tukey post hoc test with $p \leq 0.05$ in HMS and MMS; $p \leq 0.06$ in LMS; $n=24$).

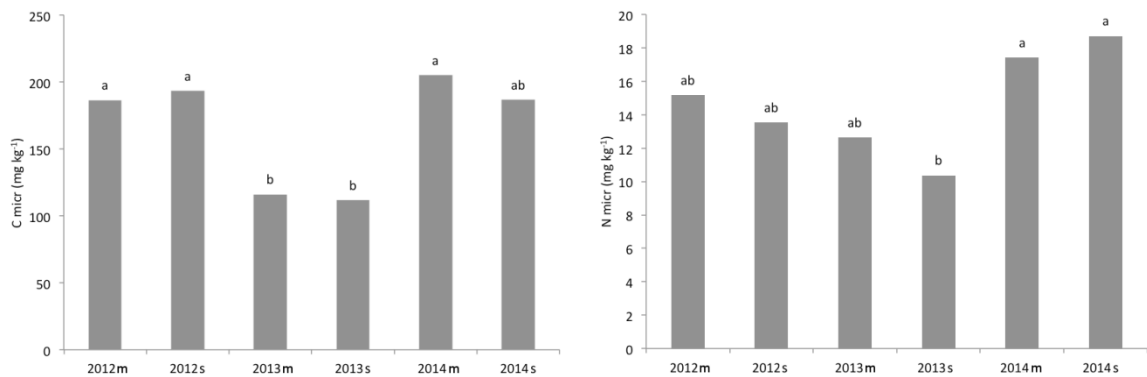


Figure 6 - Microbial biomass carbon (micr C) (left) and nitrogen (micr N) (right) concentrations during the monitoring years (m = May; s = September). Microbial C and N differ significantly when labelled with different letters (Tukey post hoc test with $p \leq 0.05$; $n=12$).

3.5 Soil organic carbon and total nitrogen stock variations

Variations in SOC stocks from 2011 to 2014 were tested on the 0-5 cm, 0-30 cm and 0-50 cm soil profiles according to the ESM method (Tab. 3). SOC stocks in 2011 were 4.03 t ha⁻¹ (0-5 cm), 22.45 t ha⁻¹ (0-30 cm) and 56.94 t ha⁻¹ (0-50cm). In the surface layer CONS yielded an increase of 0.85 t C ha⁻¹ while a decrease of 0.23 t C ha⁻¹ was observed in CONV ($p < 0.01$, Tab. 3). The overall difference between treatments was 1.08 t C ha⁻¹, corresponding to a rate of 0.36 t C ha⁻¹ y⁻¹. SOC stock variation in the first 30 cm was still positive in CONS (0.57 t C ha⁻¹) and negative in CONV (-0.08 t ha⁻¹), but the difference between the two systems was reduced to 0.65 t C ha⁻¹, 0.22 t C ha⁻¹ y⁻¹. Finally, SOC stock variation in the 0-50 cm profile was -0.69 in CONS and 1.18 in CONV, on average. However, data showed a large variability and differences between the two systems were not significant (Tab. 3).

Treatment influence on SOC stock variation was disentangled applying a linear mixed-effect model considering the residues and root biomass of crops and cover crops as continuous factors and the tillage type (no-tillage vs conventional tillage) as categorical factor. At 0-5 cm, only the treatment resulted as significant, yielding an increase of 0.89 t C ha⁻¹ in CONS and a depletion of -0.16 t C ha⁻¹ in CONV, while both texture and C input (of the crops) were ineffective. The latter were instead significant (Tab. 4) at 0-30 cm, being C positively and sand negatively correlated to SOC variation. C residue was also significant, but its quantitative influence was negligible.

Lastly, in the 0-50 cm profile no treatment effect was observed on the SOC stocks, either in terms of C input or tillage type. Tillage type was significant only to explain the C stock variation in the 30-50 cm layer, being -1.41 t C ha⁻¹ in CONS and 1.50 t C ha⁻¹ in CONV (Tab. 4).

TN stock variation in the three profiles was affected by year but not by treatment. A general depletion was observed with respect to the initial values in 2011 (0.58 t N ha⁻¹ at 0-5 cm, 3.75 t N ha⁻¹ at 0-30 cm, 7.68 t N ha⁻¹ at 0-50 cm), the variation resulting as -0.05 t N ha⁻¹, -0.43 t N ha⁻¹ and -1.03 t N ha⁻¹ at the end of the experiment (Tab. 3).

Table 3 - Soil organic carbon and total nitrogen stock variations from 2011 to 2014.

Layer	Soil organic carbon stock variation (t ha ⁻¹)			Total nitrogen stock variation (t ha ⁻¹)		
	CONV	CONS	p-value	CONV	CONS	p-value
0-5 cm	-0.23	0.85	p<0.01	-0.09	0.01	n.s.
0-30 cm	-0.08	0.57	p<0.01	-0.44	-0.41	n.s.
0-50 cm	1.18	-0.69	n.s.	-0.97	-1.08	n.s.

Table 4 - Comparison of significance level among the linear mixed-effect models analysis of soil organic carbon stock variation (Δ SOC) in 0-5 cm, 5-30 cm, 30-50 cm, 0-30 cm and 0-50 cm soil layers.

Effect	Δ SOC 0-5 cm	Δ SOC 5-30 cm	Δ SOC 30-50 cm	Δ SOC 0-30 cm	Δ SOC 0-50 cm
Intercept	0.64	0.42	0.96	0.01	0.50
Tillage	<0.01	0.58	0.04	0.31	0.21
Residue (t ha ⁻¹)	0.77	0.91	0.06	<0.01	0.20
Root (t ha ⁻¹)	0.88	0.50	0.41	<0.01	0.91
Sand(g 100 g ⁻¹)	0.10	0.59	0.94	<0.01	0.63
Clay (g 100 g ⁻¹)	0.83	0.11	0.43	0.88	0.07

3.6 Mineral composition

Clay fraction – The three analyzed farms show different clay mineral composition as revealed by their oriented specimen diffraction patterns (air-dried and EG saturated, Fig. 7).

Qualitative analysis of diffraction data of F1 shows a high content of illite and little kaolinite and chlorite. Residual quartz, calcite and dolomite were also detected in the clay fraction, whereas smectite was not detected. The clay fraction of F2 indicates the presence of kaolinite and chlorite (7.15 and 7.06 Å, 3.57 and 3.54 Å diffraction peaks respectively), illite (10.0 Å and 4.98 Å diffraction peaks), chlorite (14 Å peak unchanged after EG saturation) and a minor fraction of smectite (broad band between 11 and 13 Å shifting to 17 Å after EG saturation). F3 contains a higher amount of smectite (14 Å peak shifting to 17 Å after EG saturation), accompanied by chlorite, kaolinite, illite and serpentine (7.27 Å and 3.62 Å peaks). The residual peak at 14 Å persisting after thermal treatment at 350 °C confirms that chlorite is present and excludes vermiculite.

Bulk samples - F2 and F3 show similar bulk mineral composition characterized by prevalent quartz, feldspars, mica/illite, and minor amounts of carbonates (calcite and dolomite). Semi-quantitative estimations of phase amounts are reported in Tab. 5. Due to poor crystallinity of smectite, its direct quantification by the Rietveld method was not possible. The estimated amount of smectite in samples was measured as if it was an amorphous phase. Mineral composition of F1 strongly differs from F2 and F3, having more carbonates (dolomite, calcite) and less silicates (quartz, feldspar, mica). The amorphous content of F1 is below the detection limit of the applied method.

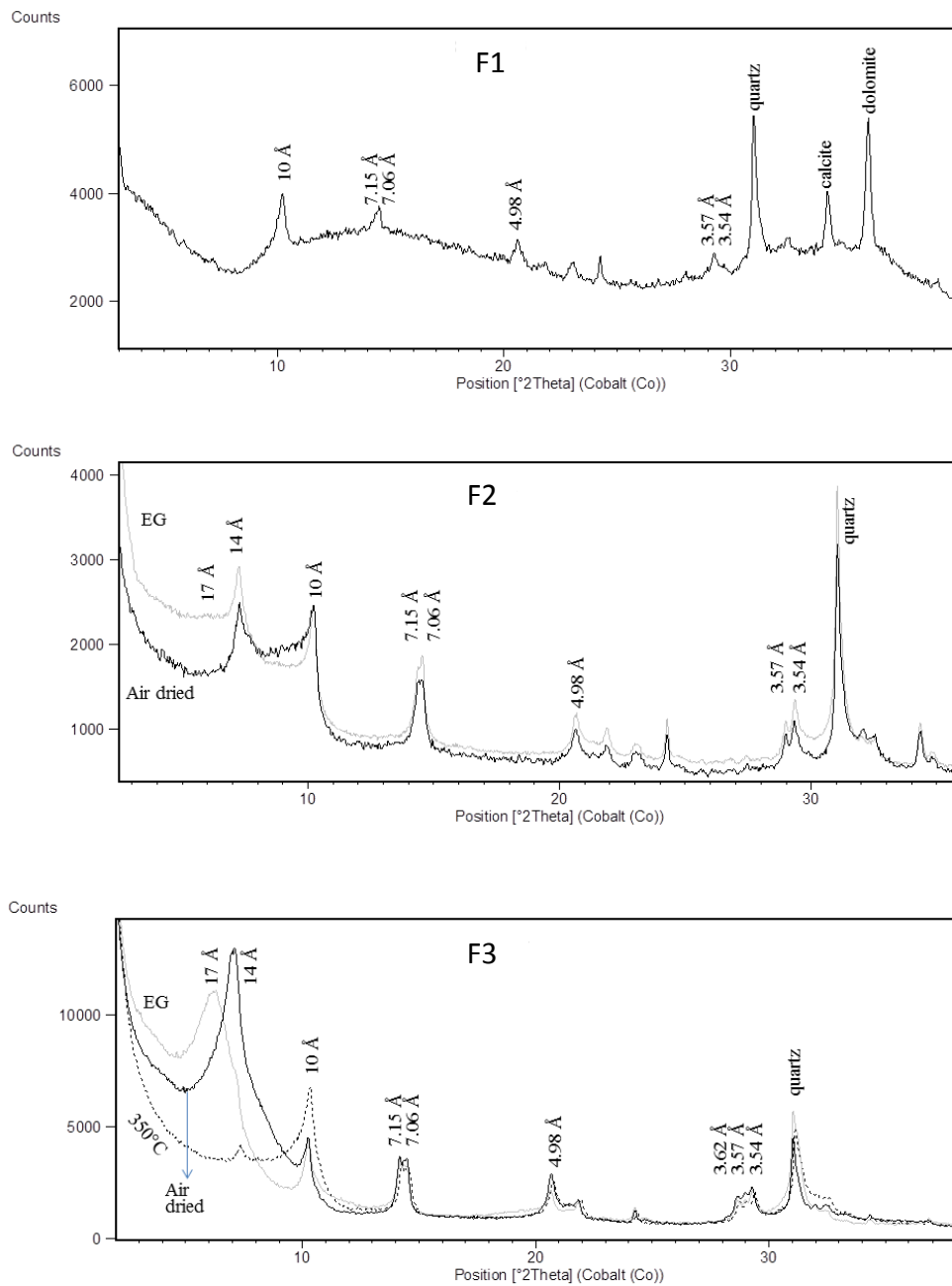


Figure 7 - Diffraction patterns of air dried and EG saturated oriented specimen of F1, F2 and F3 (heated at 350°C).

Table 5 - Estimation of weight fractions of mineral phases in bulk samples.

Phase	Farm 1 wt%	Farm 2 wt%	Farm 3 wt%
Quartz	18.3	27.3	26.0
Ms/ill	2.1	21.7	14.7
Feldspar	5.4	16.7	15.2
Chl/Kl	4.2	8.3	6.8
Calcite	21.7	3.5	3.6
Dolomite	47.5	3.7	1.7
Aragonite	1	–	–
Serpent.	–	–	2.7
Mica	–	–	1
Amphib.	–	–	0.7
Amorphous	–	18.7	27.6

Ms/ill: muscovite/illite; Chl/Kl: chlorite/kaolinite; Serpent.: serpentine; Amphib.: amphibole. Farm 1: “Vallevecchia”; Farm 2: “Diana”; Farm 3: “Sasse-Rami”.

4 Discussion

The transition from conventional to conservation system led to soil compaction in the subsurface layer (5-30 cm) as a result of the high traffic load, especially during harvesting, and the absence of tillage operations. Our results are in accordance with other studies as well as those summarized in the recent review by Palm et al. (2014). Conversely, the accumulation of crop residues on the soil surface guaranteed a lower bulk density in the top 0-5 cm layer (cf. Kay and VandenBygaart, 2002).

Soil compaction may have negatively affected other soil physical properties (e.g. porosity, air permeability) also impacting on the root growth and pattern (Lipiec et al., 2012; Mentges et al., 2016). The higher root biomass observed in the conservation system suggests a limited root penetration and more superficial root lateral development. This behaviour in plant root development has been observed previously and can be associated with excessive compaction of soil layers (Dal Ferro et al., 2014), insulating effects of surface residues altering soil temperature along the profile (Muñoz-Romero et al., 2012) or inappropriate distribution of soil water (Dwyer et al., 1996). Unfortunately, our data did not allow the root growth to be evaluated in layers deeper than 50 cm, which could be particularly relevant in the SOC stratification of the conventional system (Baker et al., 2007).

Analyses of SOC dynamics during the transition period demonstrated that the difference between the two systems was not in organic C stocks but in their depth distribution, as already emphasized by other authors (Angers and Eriksen-Hamel, 2008; Luo et al., 2010; Powlson et al., 2011). Indeed, C accumulation was observed in CONS only when the balance was accounted in the top 30 cm. The estimated C accumulation rate (0.22 t C y^{-1}) was in accordance with many other studies that restricted their monitoring activities to the top 20-30 cm depth (e.g. West and Post, 2002).

On the contrary, the balance of the whole 0-50 cm profile showed wide variability and demonstrated no potential benefit of the conservation system for C storage. The variability also confirms the methodological limitations in the detection of C changes, especially when changes are distributed over a greater soil volume (Schrumpp et al., 2011).

The effects of conservation agriculture on SOC dynamics depend on how the agronomic practices (i.e. NT, surface residues retention, crop rotations with cover crops) interact with one another and with the climate, soil type (texture and mineralogy) and nutrient availability. Crop rotations and surface residue retention are applied to increase C inputs relative to conventional practices and NT to decrease decomposition through increased soil aggregation and the protection of soil C from decomposers (Palm et al., 2014). Their application also affects the C stratification within the profile by avoiding soil inversion and influencing the root growth and pattern (Baker et al., 2007).

Crop residues together with root apparatus of crops and cover crops represented a high C input in both management systems. The retention of crop residues on the soil surface and the absence of tillage operations drove SOC dynamics in the top layer of CONS, while residue incorporation with ploughing was responsible for the SOC accumulation at 30-50 cm depth in CONV. The effect appeared to be independent of the residues biomass production that did not differ between the two systems. SOC stock variation in CONS was also driven by root C input that was identified as a major factor able to promote SOC accumulation in the 0-30 cm profile. The relevance of root C input has been emphasized by Rasse et al. (2005), who estimated that the mean residence time of root-derived C was 2.4 times that of shoot-derived C. The higher stabilization was attributed to physical protection mechanisms and only a small proportion to chemical recalcitrance.

The positive correlation found in our experiment between SOC and clay content confirmed the importance of the physical protection on C dynamics (Six et al., 2002), independently of management system. Chemical stabilization may also be influenced by clay content and mineralogy particularly in calcareous soils where Ca-bridging may play an important role on SOC persistency (von Lützow et al., 2006). This type of dependency was found only in two farms out of three, thus evidencing a potential influence of mineral composition on SOC dynamics. The specific surface area (SSA) provided by the clay minerals controls the extent to which SOM is stabilized in different soils due to mineral surfaces binding (von Lützow et al., 2006; Barré et al., 2014). The surface reactivity of the mineral constituents is higher for 2:1 than 1:1 layer type clay minerals, according to the rank montmorillonite > vermiculite > illite > kaolinite (von

Lutzow et al., 2006). Although F2 and F3 showed similar bulk mineral composition, the positive effect of clay on SOC was found only in the latter, whose clay mineral composition is characterized by a higher fraction of smectite (montmorillonite).

The peculiar composition of minerals in F1, characterized by calcite and dolomite in the clay fraction and the absence of smectite, suggests a carbonate-mediated mechanism of SOM stabilization (Baldock and Skjemstad, 2000). Carbonate could indirectly affect the stabilization of SOM by complexation of Ca^{2+} (Tipping, 2002), a process that promotes the precipitation of thin carbonate coatings, particularly on fresh residues, by metal bridging between negatively charged OM and clay mineral surfaces as well as by promoting the stability of aggregate. Recently, Fernández-Ugalde et al. (2014) found an additional mechanism of SOM protection mediated by the low-porosity induced by carbonates in soil macro-aggregates. The lower carbonate contents in F2 with respect to the other soils could also partially explain the weak correlation between SOC and clay contents.

The strong interactions existing between management systems and local soil conditions were confirmed by the C quality analyses. Soil conditions driving SOM humification processes are generally associated with more polycondensed humic molecules leading to humic extracts rich in high molecular weight size-fraction (HMS; >100 KDa) (Pizzeghello et al., 2001). On the contrary, disturbed soils with stronger mineralization processes usually have high percentages of low molecular weight size-fraction (LMS; <10 KDa) (Gerzabek et al., 1991). Our results showed different situations in terms of molecular weight distribution accounting for the variability of soil texture, soil chemical characteristics and microclimatic situation among the three farms, factors known to drive SOM dynamics in other environments. Only soils of F2 showed significant differences related to tillage, with higher MMS and lower LMS in no-tilled soils.

The complex structure of humic substances is largely responsible for their stability, although other factors such as the formation of stable clay–organic matter complexes and physical inaccessibility of organic matter within soil aggregates are also important (Haynes, 2005). Despite we did not investigate the chemical composition, the possible origin of these substances, or the interaction with soil inorganic constituents, our results suggest that less soil disturbance could have led to a better humification process and

more polycondensed humic substances (Nardi et al., 2004). They indicate also that response to soil tillage practices also depend on other site-specific environmental factors modulating the overall outcome of different soil management.

Soil microbial biomass content is another widespread soil quality indicator. Some researchers (McGill et al., 1986; Wang et al., 2012) reported that an increase in soil microbial biomass may lead to nutrient immobilization, whereas a decrease could result in the mineralization of soil nutrients. In our study soil tillage management did not affect microbial carbon and nitrogen contents. No-tillage conditions have previously been reported to show contrasting results for this parameter (Dalal, 1998; Gil-Sotres et al., 2005), so the presence of a cover crop could also have played a role. We suggest that in the no-tilled soils the balance between immobilization and mobilization still has to be reached. In a previous study on a chronosequence of untilled soils (Adl et al., 2006) microbial biomass carbon content evidenced no significant differences between control and soils untilled for 4 or 8 years.

5 Conclusions

GHG control is only one of the numerous ecosystem services provided by conservation practices (e.g. increasing of aggregates stability, reduction of erosion and P particulate loss). Many of these (e.g. aggregates stability) depend on the C content and quality of the top layer, which are strongly affected by the C stratification processes as demonstrated in this study.

Moreover, from a methodological perspective, our study confirmed the importance of performing deeper sampling and applying the equivalent soil mass method for a sound monitoring programme.

However, this research did not conclusively evaluate the benefits of conservation practices (i.e. no-tillage, surface residues retention and cover crops) on climate change mitigation, despite confirming what is reported in the literature, namely that this system of agronomic practices does not affect the SOC stocks but rather their distribution, at least in the short term. Long-term studies are therefore necessary in order to evaluate the real potential of CA, estimating either the CO₂ emission from the whole production process (i.e. Life Cycle Assessment) or the other GHG sources (i.e. N₂O, CH₄).

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Chapter III

Challenges of conservation agriculture practices on silty soils. Effects on soil pore and gas transport characteristics in North-eastern Italy*

*Piccoli I., Schjønning P., Lamandé M., Furlan L., Morari F. Challenges of conservation agriculture practices on silty soils. Effects on soil pore and gas transport characteristics in North-eastern Italy. Soil Tillage Res (submitted)

1 Introduction

Silty soils in the low-lying plain of the Veneto region (North-eastern Italy) are Calcisols and Cambisols (WRB, 2006) characterized by a low structural stability and soil organic carbon (SOC) contents, ranging from 0.005 to 0.01 kg kg⁻¹ (Dal Ferro *et al.*, 2016). They have traditionally been intensively tilled to provide a correct seedbed for crop growth. During the last 50-yr period the combination of simplified crop systems (e.g. maize monoculture), intensive tillage and lack of organic input (e.g. farmyard manure) have depleted the SOC stocks at a rate of 1.1 t ha⁻¹ y⁻¹ worsening the soil quality and increasing the GHGs emissions (Morari *et al.*, 2006).

Nowadays, no-tillage is a widespread technique among the sustainable agronomic practices, often called “conservation agriculture” (CA) when associated with crop diversification and permanent soil covering by residues retention and cover-crops (Vaneph & Benites, 2001).

As in the other European countries, application of CA practices is increasing in Veneto to reduce the production costs on the one side and allegedly to regulate and support several ecosystem services on the other side (Basch *et al.*, 2015; Kassam *et al.*, 2015). CA has also been subsidised during the two last rural development programs of Veneto Government (Regione Veneto, 2016, 2013).

CA is often associated with a number soil functions such as increasing of soil biodiversity, organic matter stocks and aggregate stability or decreasing of runoff, erosion and P losses and dioxide carbon emissions (Cavaliere *et al.*, 2009; Kay & VandenBygaart, 2002; Verhulst *et al.*, 2010). On the other hand the absence of tillage operations may impact the crop root growth through an increase in soil strength and bulk density, and reduce soil porosity and gas exchange (Dal Ferro *et al.*, 2014; Dwyer *et al.*, 1996; Lipiec *et al.*, 2006; Martínez *et al.*, 2016; Mentges *et al.*, 2016; Palm *et al.*, 2014b; Schjønning & Rasmussen, 2000).

The overall benefit of CA depends on soil type and climate. Soils with low structural stability and poor drainage are generally not suitable for no-tillage systems and can lead to a substantial reduction of crop yield (Soane *et al.*, 2012). Unstable soils, especially with low organic matter content, are subjected to higher risk of compaction (Ehlers & Claupein, 1994; Van Ouwerkerk & Perdok, 1994).

Soil air exchange with the atmosphere is one of the most important soil functions that directly impacts on crop productivity and environment. Being largely controlled by pore size distribution, pore continuity and water saturation (Blackwell *et al.*, 1990b; Hillel, 1998), air transport is strongly affected by tillage management (Martínez *et al.*, 2016; Mentges *et al.*, 2016; Schjønning & Rasmussen, 2000). Near-surface transport of gasses by mass flow is primarily controlled by air permeability (K_a) (Schjønning *et al.*, 2002; Stepniewski *et al.*, 1994), while diffusion is the process dominating gas exchanges in subsoil (Glinski & Stepniewski, 1985).

CA practices are expected to develop a more stable soil structure which would provide higher soil aeration (Horn, 2004). However the benefits connected to CA management are also texture-related.

In sandy soils, CA practices increased pores connectivity and continuity implying higher specific permeability and diffusivity, at least at shallow depth (Martínez *et al.*, 2016). In contrast, clay soils led to lower air permeability in no-tillage systems (Mentges *et al.*, 2016), which was probably due to higher water retention capacity and larger pore tortuosity of these soils (Deepagoda *et al.*, 2011). Only few studies evaluated gas transport characteristics of silt-rich and poorly drained soils. Schjønning and Rasmussen (2000) observed reduced air permeability and diffusivity where direct drilling and ploughed soil was compared in a silty soil of marine origin. In silty loess soils, Eden *et al.* (2012) observed a rather weak impact on soil structure after a long-term application of organic and mineral fertilizers. Authors concluded that “the high silt content prevented formation of a more resilient soil structure”, despite the gradient in soil organic carbon.

More research is needed to understand and optimize the potential of sustainable agronomic practices (Eden *et al.*, 2012; Farooq & Siddique, 2015; Nakajima & Lal, 2014; Thorbjørn *et al.*, 2008) especially concerning the application of CA systems. Moreover, the number of CA experiments in Europe over a wide range of soils, fertilizer applications and climate conditions with crops grown within rotations is still limited and requires expansion (Soane *et al.*, 2012).

The aim of this study was to evaluate the effect of CA practices on soil pore and gas transport characteristics in the silty soils of the Veneto low plain. The hypothesis tested

in this study is that CA could enhance soil functions related to aeration of the soil. More specifically greater organic matter content and biological activity in CA, at least at shallow depth, are hypothesized to yield higher macropore volume and connectivity, and in return improve gas exchange conditions. We studied air permeability, gas diffusivity, and derived indices of soil structure in a field experiment including four farms in which CA practices (no-tillage, cover crop and residues retention) were applied and compared to conventional intensive tillage system.

2 Materials and methods

2.1 Experimental sites

A field experiment was set up in four farms located in Veneto Region (North-eastern Italy) (Fig.1). Farm 1 (F1) was situated along the Adriatic coastline in a reclaimed environment ($45^{\circ} 38.350'N$ $12^{\circ} 57.245'E$, -2 m a.s.l.), the soil was Endogleyic Fluvic Cambisols (WRB, 2006) with a texture ranging from silty clay loam to silt loam (Table 1). The parent materials were calcareous silt sediments from Tagliamento and Piave rivers. Farm 2 (F2) was located in a low ancient plain originated from calcareous silt deposits of Brenta river ($45^{\circ} 34.965'N$ $12^{\circ} 18.464'E$, 6 m a.s.l.) and presented Endogleyic Calcisols (WRB, 2006) with a silty clay loam/silt loam texture. Farm 3 (F3) was located in a low recent plain at the Venice lagoon border ($45^{\circ}22'48.62''N$ $12^{\circ} 9'47.84''E$, 1 m a.s.l.) with Haplic Cambisols soils (WRB, 2006). The loamy texture was originated from the calcareous deposits of Brenta and Bacchiglione rivers. Farm 4 (F4) was located westward in the south low recent plain of the Po river ($45^{\circ} 2.908'N$ $11^{\circ} 52.872'E$, 2 m a.s.l.). It was characterized by a Gleyic Phaeozems (WRB, 2006) and silty clay loam or silt loam texture.

The climate in the region (years 1981-2010) was subhumid with annual rainfall around 829 mm in F1, 846 mm in F2, 859 mm in F3 and 673 mm in F4. In the median year, rainfall was highest in autumn (302, 241, 246 and 187 mm for F1, F2, F3 and F4, respectively) and lowest in winter (190, 157, 170 and 129 mm). Temperatures increased from January (average: 3.5, 3.0, 2.7 and 3.1°C respectively) to July (average: 23.3, 23.3, 23.2 and 23.6°C respectively). Reference evapotranspiration (ET_o) was 860, 816, 792 and 848

mm, with a peak in July (4.9, 4.6, 4.6 and 4.8 mm d⁻¹). ETo exceeded rainfall from May to September in F1, F2 and F3 and from May to October in F4.



Figure 1 - Experimental sites in the Veneto Region low plain, North-eastern Italy. Farms positions are marked with triangles.

Table 1 - Soil textural composition and organic carbon of experimental farms.

Characteristic	Unit	F1		F2		F3		F4	
		L1	L2	L1	L2	L1	L2	L1	L2
Sand (50-200 μm)	g 100 g ⁻¹	14.0	13.2	10.9	7.7	41.2	41.4	13.5	12.3
Silt (20-50 μm)	g 100 g ⁻¹	27.5	27.6	20.8	21.6	20.2	20.1	22.4	22.6
Silt (2-20 μm)	g 100 g ⁻¹	29.2	28.6	42.2	43.9	24.3	24.1	35.2	35.3
Clay (<2 μm)	g 100 g ⁻¹	29.3	30.6	26.1	26.8	14.3	14.4	28.9	29.8
Organic carbon	g 100 g ⁻¹	1.0	0.9	1.0	0.9	0.8	0.7	1.3	1.0

F1: farm 1; F2: farm 2; F3: farm 3; F4: farm 4; L1: 3-6.5; L2: 20-23.5 cm.

2.2 The experiment

Experimental treatments were established at each farm in 2010 in order to compare conventional “intensive tillage” (IT) and conservation agriculture (CA). Experimental fields were rectangular (about 400 m length x 30 m width) with an average size of 1.2

ha. Main management operations are shown in Table 2. IT consisted of traditional tillage practices based on mouldboard ploughing (35 cm) with crop residues incorporation followed by secondary tillage (i.e. disk harrowing) while CA included sod seeding (direct drilling), residues retention on soil surface and use of cover crops. The crop rotation (four-year) was the same in both treatments: wheat (*Triticum aestivum* L.), oilseed rape (*Brassica napus* L.), maize (*Zea mays* L.) and soybean (*Glycine max* (L.) Merr.). From 2014 a simplified three-year crop rotation wheat-maize-soybean was applied. CA, cover crops were also grown between the main crops: sorghum (*Sorghum vulgare* Pers. var. *sudanense*) during spring-summer while a mixture of vetch (*Vicia sativa* L.) and barley (*Hordeum vulgare* L.) during autumn-winter. Conversely, in the IT treatment the soil remained bare between the main crops (Table 2).

In IT, base dressing fertilizer was applied 1-2 weeks before the sowing while subsurface band fertilization was applied at the sowing in CA. In both management systems mineral fertilization was integrated by side dressing in maize (1 treatment) and wheat (2 treatments). There was no additional fertilization for cover crops while pesticide applications depended on crop requirements and were the same for both treatments. Before spring seeding, N-(phosphonomethyl) glycine was applied to suppress the winter cover crop in CA while sorghum suppression was achieved mechanically, through shredding.

Table 2 Main tillage operations and depths during one crop rotation cycle before the sampling.

Period	IT	CA
November 2012	Mouldboard ploughing (35 cm)	-
	Chisel ploughing (20 cm)	-
	Disk harrowing (10 cm)	-
	Winter wheat seeding (3 cm)	Winter wheat sod seeding (3 cm)
June 2013	Winter wheat harvesting	Winter wheat harvesting
July 2013	*	Summer cover-crop sod seeding
October 2013	*	Summer cover-crop shredding
November 2013	Mouldboard ploughing (35 cm)	-
	Chisel ploughing (20 cm)	-
	*	Winter cover-crop sod seeding
March 2014	Disk harrowing (10 cm)	-
	*	Winter cover-crop suppression†
April 2014	Disk harrowing (10 cm)	-
	Maize seeding (3 cm)	Maize sod seeding (3 cm)
May 2014	Hoeing	-
August 2014	Maize harvesting	Maize harvesting
November 2014	Mouldboard ploughing (35 cm)	-
	Chisel ploughing (20 cm)	-
	*	Winter cover-crop sod seeding
May 2015	Disk harrowing (10 cm)	-
	*	Winter cover-crop suppression†
	Soybean seeding (3 cm)	Soybean sod seeding (3 cm)
October 2015	Soybean harvesting	Soybean harvesting
November 2015	Soil sampling	Soil sampling

IT: intensive tillage; CA: conservation agriculture; - absence of tillage operation; * bare soil; † N-(phosphonomethyl) glycine based

2.3 Soil sampling

Sampling took place simultaneously in both treatments and at all four farms in October 2015 after soybean harvesting and before tillage operation in the IT treatment. In each field, three sampling plots (1 m × 5 m) were delimited, and soil samples were collected at three points and two layers, 3-6.5 cm (L1) and 20-23.5 cm (L2). Soil cores were collected by carefully removing the top soil to the intended depth, hammering sharp-edged steel cylinders into the soil, gently removing the bulk soil and fixing lids at each end. At the same positions disturbed soil samples were also collected for particle size distribution analyses. Samples were then stored at 2°C before analyses. A total of 144 undisturbed 100 cm³ soil cores (60.6 mm Ø, 34.8 mm H) and disturbed samples were collected according to the factorial combination of 4 farms × 2 layers × 2 treatments × 3 plots × 3 replicate cores.

2.4 Laboratory measurements

Disturbed soil samples were air-dried, sieved at 2 mm, dispersed in 2% sodium hexametaphosphate solution and shaken for 12 h at 80 rpm. Particle size distribution (PSD) was determined through laser diffraction method (Mastersizer 2000, Malvern Instruments).

The cores were trimmed with a knife before further analyses. Air permeability and air-filled pore space by pycnometry was measured at the field water content (FWC) as described below, and the cores next placed on sandboxes for saturation with water. Following drainage to -100 hPa matric potential, air permeability, air-filled pore space by pycnometry was measured again together with oxygen diffusion.

Air-filled porosity was measured using two methods: pycnometer (ϵ_{a_pyc}) and mass balance (ϵ_{a_mb}). The air pycnometer is based on Boyle's law and records the volume of an object inside a measuring chamber. Knowing the external volume of the sample ϵ_{a_pyc} is calculated by difference (Flint & Flint, 2002). While the pycnometer measures the connected air-filled porosity, mass-balance takes into account the entire air-filled pore space.

For each core ε_{a_mb} was calculated from volumetric moisture content (Θ) and total porosity (TP), Θ was calculated from the weight of samples before and after oven-drying at 105°C for 48 h. TP was derived from soil bulk density (BD) and particle density (PD). PD was measured on a representative sample for each farm by helium pycnometer (Micro Ultrapyc 1200e, Quantachrome, England).

Before air permeability (k_a) measurement, soil along the ring edge was gently pressed down to avoid the risk of air leaking between ring wall and soil. k_a was then measured with the steady-state method described in Iversen et al. (2001) applying 5 hPa gradient pressure from the top to the bottom of the soil core. After steady state establishment, the volumetric air flow was recorded and k_a calculated from Darcy's law.

Gas diffusion (D_s) was measured by a non-steady state method using the one-chamber apparatus described by Schjønning et al. (2013). Prior to measurements the chamber was flushed with oxygen-free N_2 . O_2 was used as the diffusing gas and its concentration was measured every 2 minutes for two hours. The diffusion coefficient was calculated from Fick's second law and then converted to gas-independent diffusivity (D_s/D_o) dividing by oxygen diffusion coefficient (D_o) in free-air ($0.205 \text{ cm}^2 \text{ s}^{-1}$ at atmospheric pressure and 20°C).

In F1, F2 and F4 more than 2 mm of swelling were recorded and as a result of non-precise measurements of samples height, parameters including soil volume (ε_{a_pyc} , ε_{a_mb} , SP and SD) were excluded from this work with the exception of F3 in which swelling was not observed.

2.5. Calculations

Degree of compactness (DC) was calculated according to Keller and Håkansson (2010):

$$DC = 100 \frac{BD}{BD_{ref}} \quad [1]$$

where BD is the bulk density (g cm^{-3}) and BD_{ref} (g cm^{-3}) is reference bulk density. BD_{ref} is obtained by 24 h uniaxial compression test at 200 kPa stress (Håkansson & Lipiec, 2000). In this study, BD_{ref} was estimated according to equation n. 13 of Keller and Håkansson (2010):

$$BD_{ref} = 1.508 + 0.226 \log \alpha + 0.417 \beta + 0.110 \beta^2 - 0.0242 OM - 0.0110 OM \log \alpha$$

[2]

where α and β are parameters obtained by fitting the Rosin-Rammler function to the PSD (Rosin & Rammler, 1933). α (μm) represents the particle size corresponding to the 63.22nd percentile of the cumulative probability distribution (50th percentile of normal distribution) and reflects the ‘coarseness’ of the soil. The β variable is the standard deviation of a normal distribution (logarithmic size scale) and hence provides a measure of the ‘sortedness’ of the primary soil particles (Perfect *et al.*, 1993). Soil organic matter content (OM) used for calculations were derived from soil organic carbon contents multiplying by 1.72.

Relative diffusivity (D_s/D_o) observations were compared with estimated values by the Moldrup *et al.* (2000) model for -100 hPa matric potential:

$$\frac{D_s}{D_o} = 2\varepsilon^3 a_{mb} + 0.04\varepsilon_{a_mb} \quad [3]$$

while the effective pore diameter (d_B) was calculated based on the tube model of Ball (1981):

$$d_B = 2 \left(\frac{8k_a}{D_s/D_o} \right)^{1/2} \quad [4]$$

where ε_{a_mb} is the air-filled porosity by mass balance calculation and k_a the air permeability. For each sample, specific air permeability (SP) and diffusivity (SD) were calculated dividing k_a and D_s/D_o by ε_{a_mb} .

2.6 Statistical analysis

Air permeability and effective pore diameter data were log-transformed before analyses.

For each layer, data were analysed with a linear mixed-effect model based on REML (Restricted Maximum Likelihood) estimation method, considering treatment, farm and farm \times treatment interaction as fixed and plot as random effect. Post-hoc pairwise comparisons of least-squares means (LSE) were performed using Fisher LSD method at significance level < 0.05 . Root mean square error (RMSE), bias and t-test were used to

compare relative gas diffusivity observations and estimated values. Statistical analyses were performed with SAS software (SAS Institute Inc. Cary, NC, USA), 5.1 version.

3 Results and discussion

3.1 Bulk density

Bulk density (BD) measured at FWC in the upper layer (L1) was affected by treatment and farm (Table 3), with higher values in CA (1.58 g cm^{-3}) than IT (1.48 g cm^{-3}), and in F1, F3 and F4 than F2. For the deeper layer (L2), BD was larger in CA than IT (1.67 vs 1.49 g cm^{-3}) only in F3, as indicated by a significant interaction farm \times treatment, while on the average for the other farms was 1.65 g cm^{-3} , irrespective of treatments (Table 3). This is in accordance with other studies (Dal Ferro et al., 2014; Piccoli et al., 2016; Schjønning and Rasmussen, 2000; Yang et al., 1999), where soil compaction was observed in silt-rich soils subjected to CA treatment. This compaction could be due to traffic, especially during the harvesting operations, as also observed for Danish sandy loam by Munkholm et al. (2003). Silty soils usually show a low resilience to structural damages (Eden *et al.*, 2012). Soil compaction was largest for F3 than for the other farms due to coarser texture and very lower organic carbon content. Indeed coarser soils are more prone to compaction (Keller & Håkansson, 2010; Schjønning & Thomsen, 2013) due to the not self-mulching behaviour (Ehlers & Claupein, 1994).

The degree of compactness (DC) in the farms ranged from 95 to 104% in L1 and from 104 to 109% in L2 under CA while from 89 to 100% in L1 and from 89 to 109% in L2 under IT (Table 4). Higher values were estimated under CA treatment in the upper layer of F1, F3 and F4 and in the lower layer of F3. Irrespectively of the treatment, most of values exceeded 87% (Table 4) which was reported as optimal DC for crop growth (Håkansson, 1990) by affecting soil strength and air-filled porosity. The densities observed for nearly all combinations of farm and soil layer in this study are thus way higher than the optimal for agricultural use (Naderi-Boldaji and Keller, 2016).

Benefits from organic matter accumulation on soil surface such as the improvement of soil structure were not observed after 5-yrs of CA management. Most likely in silty soils longer periods are required to attain a new soil equilibrium and in turn exploit the

benefits provided by conservation agriculture practices (Soane et al., 2012; Vogeler et al., 2009).

Table 3 - Estimated mean, statistics and standard error (SE) for studied characteristics.

Different letters indicate significant difference between estimated means according to Fisher LSD test with $p < 0.05$

Layer (cm)	Effect		BD ¹ (g cm ⁻³)		ka ² (μm ²)		D _s /D _o ²		d _B ² (μm)			
			Mean	SE	Mean ³	SE ⁴	Mean	SE	Mean ³	SE ⁴		
3-6.5	Treatment	p-value	0.0013		0.7671		0.0029		0.0179			
		CA	1.58	a	0.02	3.93	(-0.10)	0.0058	0.0009	184.33	a (-0.04)	
		IT	1.48	b	0.02	3.44	(-0.17)	0.0124	0.0018	127.61	b (-0.04)	
	Farm	p-value	0.0071			0.1578		0.1895		0.2301		
		F1	1.53	a	0.02	4.88	(-0.14)	0.0089	0.0018	157.14	(-0.03)	
		F2	1.45	b	0.02	5.37	(-0.20)	0.0083	0.0019	194.72	(-0.08)	
		F3	1.55	a	0.03	4.8	(-0.11)	0.0125	0.0027	139.35	(-0.04)	
		F4	1.58	a	0.03	1.46	(-0.27)	0.0066	0.0021	129.78	(-0.07)	
	Farm x treatment	p-value	0.1937			0.3033		0.0171		0.9048		
		F1CA	1.57		0.03	9.68	(-0.13)	0.0094	b	0.0022	201.84	(-0.02)
		F1IT	1.49		0.03	2.46	(-0.22)	0.0085	b	0.003	122.35	(-0.03)
		F2CA	1.46		0.03	4.1	(-0.32)	0.0064	b	0.0024	221.21	(-0.14)
		F2IT	1.44		0.04	7.03	(-0.26)	0.0103	b	0.0028	171.4	(-0.05)
		F3CA	1.64		0.02	3.17	(-0.06)	0.0036	b	0.0004	173.34	(-0.04)
		F3IT	1.47		0.03	7.27	(-0.19)	0.0215	a	0.0034	112	(-0.06)
		F4CA	1.63		0.03	1.91	(-0.17)	0.0039	b	0.0013	149.11	(-0.04)
F4IT	1.53		0.05	1.11	(-0.53)	0.0093	b	0.0039	112.95	(-0.14)		
20-23.5	Treatment	p-value	0.1842		0.5507		<.0001		0.7002			
		CA	1.65		0.02	1.23	(-0.11)	0.0028	0.0004	143.62	(-0.03)	
		IT	1.62		0.02	1.59	(-0.15)	0.0063	0.0014	152.34	(-0.05)	
	Farm	p-value	0.0524			0.0109		<.0001		0.4631		
		F1	1.63		0.01	3.42	(-0.12)	0.0046	0.0006	169.24	(-0.03)	
		F2	1.63		0.02	0.78	(-0.21)	0.0016	0.0004	159.59	(-0.08)	
		F3	1.58		0.03	2.92	(-0.15)	0.0109	0.0023	122.69	(-0.02)	
		F4	1.68		0.01	0.49	(-0.19)	0.0011	0.0003	144.41	(-0.07)	
	Farm x treatment	p-value	0.0186			0.0145		<.0001		0.7265		
		F1CA	1.64	a	0.01	4.93	b (-0.09)	0.0055	b	0.0008	175.55	(-0.02)
		F1IT	1.63	a	0.02	2.37	bc (-0.21)	0.0038	bc	0.0009	163.12	(-0.06)
		F2CA	1.62	a	0.03	0.51	bc (-0.32)	0.0016	cd	0.0007	138.52	(-0.09)
		F2IT	1.64	a	0.03	1.18	bc (-0.28)	0.0015	cd	0.0004	183.87	(-0.14)
		F3CA	1.67	a	0.01	0.81	c (-0.08)	0.0022	cd	0.0003	112.75	(-0.03)
		F3IT	1.49	b	0.03	10.51	a (-0.08)	0.0196	a	0.0019	133.48	(-0.03)
		F4CA	1.66	a	0.02	1.12	c (-0.13)	0.0018	cd	0.0005	155.1	(-0.03)
F4IT	1.7	a	0.02	0.22	bc (-0.36)	0.0004	d	0.0001	134.46	(-0.17)		

BD: bulk density; k_a: air permeability; D_s/D_o: relative diffusivity; d_B: effective pore diameter; IT: intensive tillage; CA: conservation agriculture; ¹ field water content (FWC); ² -100 hPa matric potential; ³ geometric mean; ⁴ standard error of log-transformed mean (in brackets).

Table 4 - Bulk density, reference bulk density and degree of compactness as affected by treatments and layers in the experimental farms.

Farm	Bulk density (g cm ⁻³) ^a				Reference bulk density (g cm ⁻³) ^b				Degree of compactness (%) ^c			
	3-6.5 cm		20-23.5 cm		3-6.5 cm		20-23.5 cm		3-6.5 cm		20-23.5 cm	
	CA	IT	CA	IT	CA	IT	CA	IT	CA	IT	CA	IT
F1	1.57	1.49	1.64	1.63	1.49	1.48	1.49	1.50	104	100	109	108
F2	1.46	1.44	1.62	1.64	1.46	1.44	1.45	1.44	95	95	106	108
F3	1.64	1.47	1.67	1.49	1.65	1.66	1.62	1.67	100	89	104	89
F4	1.63	1.53	1.66	1.70	1.48	1.45	1.48	1.47	104	99	105	109

^aBulk density calculated with core method (Grossman & Reinsch, 2002a) at field water content (FWC)

^bReference bulk density calculated by equation n. 13 in Keller and Håkansson (2010)

^cDegree of compactness calculated by equation n. 1 Keller and Håkansson (2010)

3.2 Air filled-porosity

Soil swelling after equilibration at -100 hPa was observed in three out of four farms. For this reason air-filled porosity was measured only for the no-swelling soil of farm 3. Observations obtained with the two methods applied (pycnometry and mass-balance calculations) were in agreement at -100 hPa but not at field water content (FWC) (Fig.2). Following saturation of the samples and drainage to -100 hPa in the laboratory, observations were very close to the 1:1 line, suggesting no or very few blocked air-filled pores. This is in accordance with a previous study on a silt loam soil deriving from loess soil (Eden et al. 2012). In contrast, at FWC, ε_{a_pyc} was systematically lower than ε_{a_mb} , (regression slope 1.08 and intercept -0.20 m³ m⁻³) suggesting the occurrence of air-filled porosity blocked from or not connected to the surrounding atmosphere. We note that this estimate of air-filled pores not in contact with the surrounding atmosphere was about 0.18 m m⁻³ and not affected by tillage or sampling depth. These results suggest that a different water dynamics occurred in field compared to the laboratory conditions. In the latter, soil sample preparation was standardized and consisted in the soil saturation by imbibition followed by drainage at -100 hPa. On the contrary, field water conditions reflect composite processes where wetting (e.g. infiltration) and drying (e.g. evapotranspiration) occurred simultaneously or sequentially, generating the encapsulation of air in non-connected pores. Water dynamics are hysteretic and natural

water distribution in unsaturated soils shows a high spatial variability (Martello *et al.*, 2015). Fig. 2 shows that natural water distribution in soils can be quite different from water distribution after saturation and drainage in the laboratory. Thus, conclusions derived from gas transport measurements on soil cores equilibrated to a given matric potential should be carefully transferred to field conditions. In the present work, measurements depicted a situation with no or reduced blocked air-filled porosity, which represent an ideal case for both treatments. This means that the conditions for aeration of the soils in the field may be worse than estimated in the following sections.

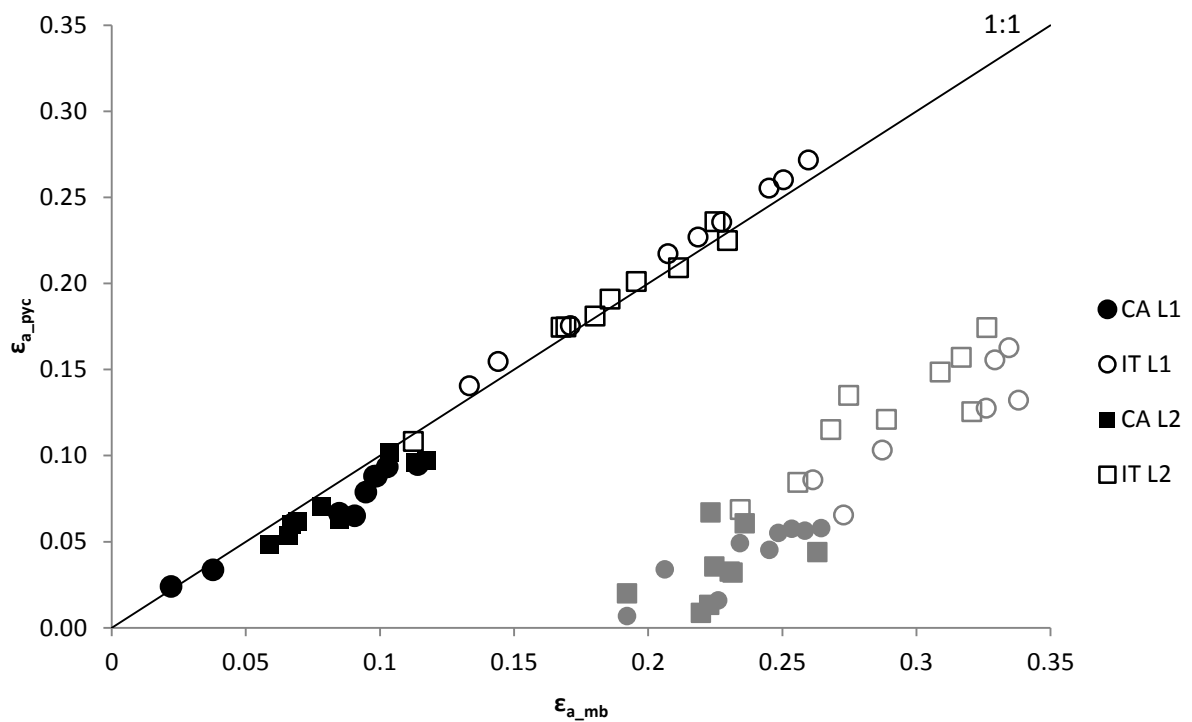


Figure 2 - Scatter plot of air-filled porosity from pycnometer (ϵ_{a_pyc}) and mass-balance (ϵ_{a_mb}) of farm 3. Grey symbols: field water content (FWC); black symbols: -100 hPa matric potential. 1:1 line is shown. CA: conservation agriculture; IT: intensive tillage; L1: 3-6.5 cm; L2: 20-23.5 cm.

3.3 Air-permeability

There were no effects of treatment or farm on air-permeability (k_a) in the upper soil layer (Table 3). K_a was significantly lower for CA than for IT at 20 to 23.5 cm depth but only at F3 (0.81 vs 10.51 μm^2) (Table 3). Air-permeability was generally low, as 82% (for

L1) and 95% (for L2) of measurements corresponded to the “low air permeability class” as defined by Fish and Koppi (1994) ($k_a < 20 \mu\text{m}^2$) suggesting poorly aerated conditions independent of agronomic practices. Air impermeability conditions ($k_a < 1 \mu\text{m}^2$; Ball et al., 1988) were recorded in 19% and 34% of samples for L1 and L2 respectively. Similar results were obtained by Arthur et al. (2013) and Eden et al. (2012) measuring the air-permeability in silt-rich soils. The slow transmission properties could lead to scarce aeration and represent a limiting factor for both crop growth and soil biological activities, especially during wet conditions. Indeed, particularly during the winter-spring months (November-May), the water matric potential in the farms soil profiles is often higher than -100 hPa (Morari *et al.*, 2012) leading to critical soil aeration.

3.4 Gas diffusivity

3.4.1 Validation of measurements

Measured relative gas diffusivity (D_s/D_o) was compared with the one estimated through the model presented by Moldrup et al. (2000) in both normal and log-transformed values (Fig.3). Despite p-value t-test was significant, the measurements seem to be in line with those from a range of other soils as verified by the model predictions. The fitting was not affected by treatment or layer while lower values were associated with CA treatment (Fig.3) and confirmed model estimation.

3.4.2 Measured gas diffusivity

The relative gas diffusivity was higher in IT than CA for both the layers (Table 3). Results were dominated by conditions observed in F3 (Table 3) where CA practices decreased relative gas diffusivity of 0.0179 in L1 and 0.0174 in L2 (interaction treatment x farm significant at $p < 0.05$; Table 3). These results may be a consequence of the tillage effect on soil bulk density (Eden et al., 2012). Indeed soil compaction has been demonstrated to affect negatively gas diffusivity by increasing tortuosity and decreasing pores continuity (Berisso *et al.*, 2013; Schjønning & Rasmussen, 2000).

On average, soils of the Veneto low plain showed a poor relative diffusivity with values below the threshold of 0.005 representing the lower limit for the aerobic microbial activity (Schjønning *et al.*, 2003). Specifically 64% (CA) and 42% (IT) of the data in L1 and 81% (CA) and 72% (IT) in L2 were under microbial anaerobic conditions as judged from the above criterion. These results indicate potential conditions for high denitrification activities, not only in the quasi-saturated zone above the ground water but also at shallower layers. Rochette (2008) found in poor aerated soils under no-tillage management an increase of 2 Kg N ha⁻¹ in N₂O emissions, which offset the benefits of no-tillage system in terms of soil C sequestration.

The scale of investigation of this study could not allow measuring the effects of big cracks, which can be responsible for oxygen diffusion in subsoil (Bear, 1972; Iversen *et al.*, 2012). Moreover, equilibrated samples in laboratory do not represent the complexity of air-filled pore space condition observed at field scale. Indeed encapsulation of air in not-connected pores could be neglected by laboratory analyses. However in the field conditions, at the micro scale, the above results indicate that hotspots with anoxic and denitrification conditions seem likely for these soils, especially under CA practices.

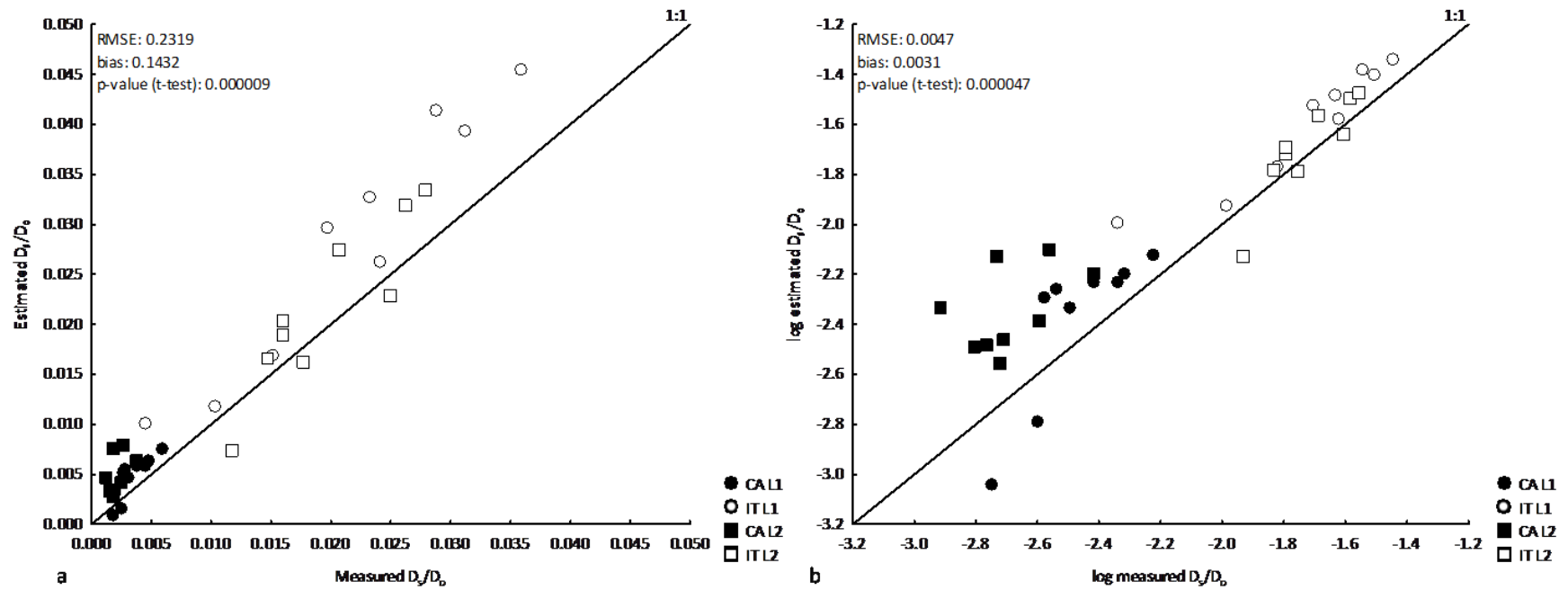


Figure 3 - Scatter plots of estimated (Moldrup *et al.*, 2000) and measured relative diffusivity (D_s/D_o) normal distributed (a) and log-log distributed (b) for soil samples collected at the F3 farm and drained to a matric potential of -100 hPa. 1:1 line and statistics are shown. CA: conservation agriculture; IT: intensive tillage; L1: 3-6.5 cm; L2: 20-23.5 cm.

3.5 Effective pore diameter

Effective pore diameter (d_B) was affected by treatment in L1 being higher in CA (184 μm) than IT (128 μm , Table 3). In L2 d_B was on average 148 μm and not significantly affected by tillage (Table 3).

Pore networks with larger size with respect to the conventional management were often observed in CA stabilized systems, after a transition period of at least 3 years (Soane and Ball, 1998). Indeed conservation practices allow the formation of large biopores mainly due to greater earthworm activity (Soane et al., 2012; Vogeler et al., 2009). These results should have led to an increase of air and water transport parameters for CA. However, a larger effective pore diameter was not related to e.g. a larger air permeability or diffusion in the present study. It indicates that effective pore diameter alone is not sufficient to understand effects of tillage treatments on soil transport parameters. In fact, the d_B variable is a ‘tool’ to quantitatively evaluate, whether large, continuous pores are dominating. Total pore volume, pore distribution, tortuosity and connectivity should also be quantified to better evaluate effects of agricultural systems on pore network functioning. A previous work conducted by Dal Ferro et al. (2014) in F3 aimed to evaluate the effects of tillage management on soil structure by mean of a combination of X-ray microtomography (microCT) and mercury intrusion porosimetry (MIP). This work showed that no-tillage did not affect the total porosity in the range 54-2250 μm but caused a shift in pore distribution toward the large classes (e.g. 250-500 μm). However, average pore diameter was larger for ploughing (390 μm) than no-tillage (320 μm).

3.6 Specific air permeability and relative diffusivity

Specific air permeability (SP) and diffusivity (SD) are useful descriptors of pore system (Blackwell *et al.*, 1990b; Gradwell, 1960). Fig. 4 presents SP and SD against ϵ_{a_mb} for F3 only (no swelling soil). In L1, despite the lower ϵ_{a_mb} and k_a , CA showed similar SP as IT while in L2, CA negatively affected both ϵ_{a_mb} , k_a and SP. On the other hand SD was negatively affected by CA practices in both layers.

In other words, CA reduced the air-filled pore volume and the relative diffusivity of the upper soil layer compared to IT, but the pores retained their ability to conduct air by convection (identical SP). In L2, the CA-induced reduction in ϵ_{a_mb} gave rise to a ~10-fold reduction in k_a but also to a reduction of the ability of this pore volume (SP) to conduct both the convective and diffusion air flow. Provided identical pore characteristics, diffusivity is determined by the pore space available to the diffusion process. The CA-induced reduction in SD thus indicates that CA not only affects ϵ_{a_mb} and the relative diffusivity but also continuity and/or tortuosity characteristics.

In the upper topsoil, biological activity and residues retention allowed the development of a more continuous pore system, and air-filled porosity retained its efficacy to conduct air- and water flow. It was not the case for the lower topsoil that remained untilled in CA, which was characterized by denser soil matrix and disconnected/tortuous pore system. Such contrasted depth functioning has been observed by Eden et al. (2011) and Schjøning and Thomsen (2013). MicroCT visualization and quantification of large pores carried out by Dal Ferro et al. (2014) showed that pore connectivity in the shallower layer was higher in the no-tillage treatment while conventional tillage disrupted the pore connectivity and reduced the pore branch length. In opposite, pore connectivity was higher for conventional tillage between 10 and 20 cm depth.

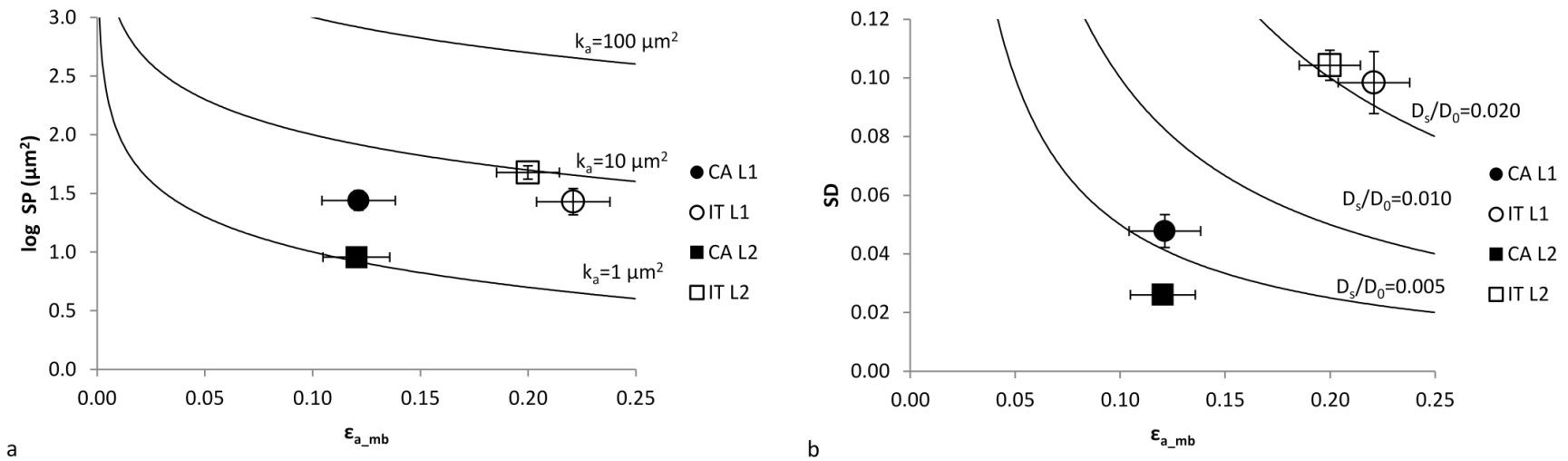


Figure 4 - Average values (\pm standard error) of log-transformed specific permeability (SP) (a) and specific relative diffusivity (SD) (b) vs air-filled porosity from mass-balance (ϵ_{a_mb}) at -100 hPa matric potential, as affected by treatment (CA: conservation agriculture and IT: intensive tillage) and depth (L1: 3-6.5 and L2: 20-23.5 cm) in farm 3. Permeability isolines equal to 1, 10 and 100 μm^2 are shown in SP graph while relative diffusivity isolines equal to 0.005 (lower limit for aerobic microbial activity according to Schjønning et al., 2002), 0.010 and 0.020 in SD one.

3.7 General discussion

Our studies of the four silty soils from the Veneto region indicated that they were generally dense with poor conditions for air exchange by convection as well as diffusion. We further noted that conservation agriculture (CA) including an increased input of organic residues had generally not been able to improve the structural conditions compared to intensive tillage (IT). Here we will give a few considerations on the mechanisms likely to explain our findings.

We hypothesized that stable soil structures also resilient to (mechanical) impacts require one or more of the following characteristics:

- a particle size distribution (PSD) with a limited inherent tendency to form dense layers when subjected to mechanical impact;
- a reasonably high content of clay minerals;
- a certain amount of organic matter.

Fig. 5 gave the characteristics of the particle size distribution for the four soils according to the Rosin-Rammler variables together with some selected soils from the literature. The contour background in Fig. 5 displayed the anticipated 'reference bulk density' (BD_{ref} (Eq. [2]) which is an expression of the tendency of the soils to densify when subjected to a mechanical load (Håkansson, 1990). We note from Fig.5 that the soils of F1, F2 and F4 were located at small α -values and relatively high β -values. I.e., these soils were fine and sorted. In Fig. 5, the F3 soil was located at higher α and lower β . Different depositional processes in the Veneto low plain could have caused the observed heterogeneous PSD distributions. In F3, the proximity to the Venice lagoon rim led to a coarser texture (littoral sand bars) while in the other farms, alluvial deposits were dominated by a finer PSD due to the lesser kinetic energy of rivers over minimal slopes ($<0.1\%$) (Costantini & Dazzi, 2013). We note that the F3 soil was the only one of the four soils studied that i) did not swell, and ii) responded to some extent to the contrasting tillage treatment. In Fig. 5, the PSD of the Veneto soils were also put in a context by a range of soils from the literature. The Jyndevad and Dronninglund soils both are located in Denmark, developed on sediments in water, the former from glaciofluvial deposits,

the latter from sediments in the Holocene Sea (Schjønning & Thomsen, 2013). The sorting action of the water provided both of these soils with high β -values, but especially the Jyndevad soil was very coarse. The Bramstrup and Kløvested soils were both morainic deposits and characterized by small β -values, reflecting their lack of a sorting procedure in their genesis (Schjønning & Thomsen, 2013). We also included three more soils with a fine texture (low α -values). The Tuscany and Sicily clay soils developed from a Palaeogene marine deposition on marly calcareous and limestone, respectively (Costantini & Dazzi, 2013) while the Bad Lauchstädt soil is a wind-deposited loess soil from Germany (Eden *et al.*, 2012). All these soils have similar coarseness as F1, F2 and F4 but display a more graded texture (lower β) than these soils. The contours of BD_{ref} indicate that the tendency to densification is highest for graded, coarse soils (upper left corner of Fig. 5). This is accordance with general experience, the Bramstrup and to some extent the Kløvested soil being typical hard-setting soils. In contrast – as based on the predicted BD_{ref} – the Veneto soils (especially the F1, F2 and F4 soils) would not be expected to densify to very high densities (Fig. 5). However, our results have shown that the bulk densities monitored in the fields of the four Veneto soils were very high relative to BD_{ref} (Table 4). The very high DC for the Veneto soils nearly irrespective of tillage treatment may relate to either issue 2 or 3 in our hypotheses above. Table 5 shows selected characteristics for the same soils as depicted in Fig.5. We note that the four Veneto soils, Tuscany, Sicily and Bad Lauchstädt soils have very high contents of particles less than 20 μm (Fines20). This is in contrast to the Danish soils irrespective of the geological origin and their PSD (Table 5). Further, especially the Italian soils display very low contents of organic carbon (Table 5). The combined effect of these characteristics can be found by the Fines20/SOC ratios. Hassink (1997) found that the amount of organic carbon that can be adsorbed by the mineral matrix of a soil is determined by the soils' content of mineral particles less than 20 μm . All Veneto region soils and the Tuscany soil display high ratios between Fines20 and SOC (Table 5). A high Fines20/SOC ratio may imply that i) there is only a limited amount of non-complexed organic carbon available for interaction with the clay minerals in structural stabilization and also for securing a resilient soil structure (Schjønning *et al.*, 2012) and ii) this soils are theoretically able to complex-bind a large quantity of SOC. We though

note that studies have demonstrated the Tuscany soil to be structurally stable (Pellegrini personal communication). This is despite its high Fines₂₀/SOC ratio (Table 5). The explanation may be that the relative amount of fine silt to clay is very low (0.60, Table 5) for this soil. It can be hypothesized that here, the organic carbon available is actually able to fulfil its role in interaction with clay for structural stabilization.

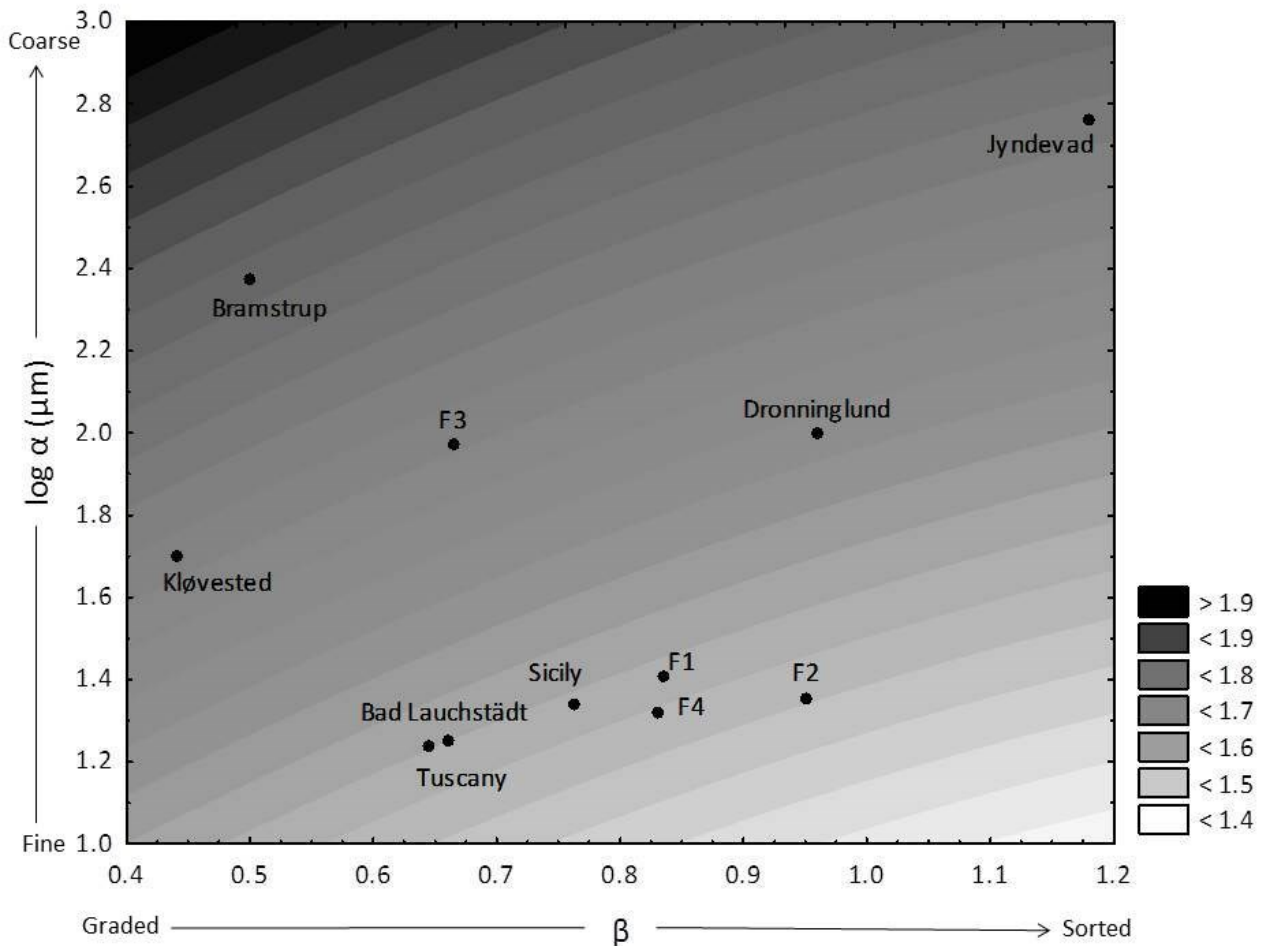


Figure - 5 Scatter plot of α and β parameters of Rosin-Rammler function. Comparison between studied farms (F1, F2, F3 and F4), two Italian clayey soils (Tuscany and Sicily) and some experimental sites reported by Schjønning and Thomsen (2013). The contour background displays the ‘reference bulk density’ (BD_{ref}) estimated by the pedotransfer function (Eq. [2]) provided by Keller and Håkansson (2010). Calculations considered soil organic matter content equal to 1%.

Table 5 - SOC, clay, silt20 and fines20 contents and silt20/clay and fines/SOC ratios of studied soils in comparison with some selected from the literature.

Soil	SOC (g 100g ⁻¹)	Clay <2 (g 100g ⁻¹)	Silt20 2-20 (g 100g ⁻¹)	Fines20 <20 (g 100g ⁻¹)	Silt20/ Clay	Fines20/S OC
F1	1.0	29.3	29.2	58.5	1.00	59
F2	1.0	26.1	42.2	68.3	1.62	68
F3	0.8	14.3	24.3	38.6	1.70	48
F4	1.3	28.9	35.2	64.1	1.22	49
Tuscany	0.9	54.8	33.1	87.9	0.60	98
Sicily	2.0	37.9	49.9	87.8	1.32	44
Bad Lauchstädt	2.2	25.6	25.4	51.0	0.99	23
Jyndevad	2.2	4.7	3.0	7.7	0.64	4
Dronninglund	6.2	10.1	10.4	20.5	1.03	3
Bramstrup	1.0	12.8	12.3	25.1	0.96	25
Kløvested	2.5	24.3	24.5	48.8	1.01	20

SOC: soil organic carbon; Fines20: particles below 20 µm.

4 Conclusions

- Air-filled porosity measurement highlighted that encapsulation of air in not-connected pores could occur at field water content, on the contrary, laboratory standardization of sample allowed the removal of most of the blocked air-filled pores; for these reasons conclusions derived from gas transport measurements on soil cores equilibrated to a given matric potential should be carefully transferred to field conditions
- Soil compaction was observed in silt-rich soils subjected to CA treatment and was particularly severe in the farm dominated by coarser texture and lower organic carbon content
- Gas transport measurements highlighted low transmission properties of silty soils of Veneto low plain independently from agronomic management, leading to critical values for both soil aeration and microbial aerobic activity
- The implementation of conservation agriculture practices is not able to easily improve soil structure despite the fact that more organic residues are actually added to the soil
- Periods longer than five years are most likely required to attain a new soil equilibrium and in turn exploit the benefits provided by conservation agriculture management
- More studies elucidating the mechanisms of highest importance in improving soil structural conditions for silty soils as those examined in this study are requested

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Chapter IV

Conservation agriculture had a poor impact on the soil structure of Veneto low-lying plain silty soils after a 5-yr transition period*

*Piccoli I., Camarotto C., Lazzaro B., Furlan L., Morari F. Conservation agriculture had a poor impact on the soil structure of Veneto low-lying plain silty soils after a 5-yr transition period. Land Degradation & Development (submitted)

1 Introduction

Soil structure is a key tracer of the changes in soil quality and soil erodibility (Pulido Moncada et al., 2015) and can inform of the degradation of the soil system (Muñoz-Rojas et al., 2016). Intensive tillage has been known to negatively affect the soil structure properties by breaking up aggregates and exposing organic matter to microbial attacks (Six et al., 1998). Conservation agriculture (CA), by including minimum soil disturbance, permanent soil covering and crop diversification (Farooq & Siddique, 2015), was demonstrated to improve soil structure and as a consequence reduce erosion, runoff, P particulate loss and CO₂ emissions (Soane et al., 2012).

Despite these benefits, negative effects on soil physical properties were also observed in CA systems (Palm et al., 2014). Subsurface compaction (higher bulk density) and greater soil strength (e.g. penetration resistance) were often related to a high traffic load, especially during harvesting operations (Munkholm et al., 2003). Soil compaction negatively affects the porosity and other physical properties such as water content distribution and gas exchanges, and in turn also the crop root growth (Dal Ferro et al., 2014; Dwyer et al., 1996; Lipiec et al., 2012).

Porosity is the best indicator of soil structure and can be measured at different scales combining different techniques. Dal Ferro et al. (2014) and Pituello et al. (2016) successfully coupled mercury intrusion porosimetry (MIP) and x-ray computed tomography (x-ray μ CT) to study soil porosity from ultramicro- to macro-scale.

MIP, even if disruptive, is a widely used technique for microstructure (<50 μ m) studies (Romero & Simms, 2008) while x-ray μ CT has copiously demonstrated to be one of the best non-destructive techniques in soil structure studies at macroscale in terms of both pore quantification and architecture (Dal Ferro et al., 2013; Pituello et al., 2016; Schjønning et al., 2013; Taina et al., 2008)

Microstructural soil studies usually focus on particles aggregation scale (<100 μ m) while macrostructural studies on higher structure levels (>100 μ m) (Romero & Simms, 2008). Although most natural air and water dynamics occur at macroporosity scale, many macroscopic behaviours can be explained by microstructural properties (Romero & Simms, 2008).

CA is usually recognized to increase total porosity (TP) in upper layers and decrease it in deeper ones as a result of higher soil organic matter content and bulk density respectively (Kay & VandenBygaart, 2002). Apart from TP, different pore size classes fulfil different roles in aeration, infiltration, drainage and storage of water and implied different mechanical resistance offered to root growth (Kay & VandenBygaart, 2002). At macroporosity level Dal Ferro et al. (2014) observed that tillage operations reduced pores $>250 \mu\text{m}$ into smaller ones (54-250 μm). On the contrary Anken et al. (2004) highlighted significant lower macroporosity in CA compared to ploughing tillage in the 5-8 cm layer. At microporosity level, Dal Ferro et al. (2014) and Lipiec et al. (2006) did not find any significant difference between CA and conventional tillage whereas Pagliai et al. (2004) showed a positive influence of conservation tillage practices on the 0.5-50 μm porosity class.

Different tillage and agronomic systems affect not only TP and pore size distribution (i.e. pore dimensions) but also pore architecture (i.e. fractal dimension, connectivity density and degree of anisotropy) and morphology (i.e. pore shape). Many authors (e.g. Dal Ferro et al., 2014; Pagliai et al., 2004) observed that conventional tillage system reduced pore branch length, pore connectivity and decreased the proportion of transmission pores. Instead, CA showed a more vertically oriented macroporosity (root channels and earthworm holes) contributing to soil aeration, water intake and moisture retention (Soane et al., 2012; Vogeler et al., 2009). Much of the research investigating different tillage-related effects on soil structure has focused on medium- to coarse-textured soils, but more information is required on fine-textured soils (Kay & VandenBygaart, 2002).

In a previous study conducted on the silty soils of the low-lying Venetian plain, Piccoli et al. (2016b) observed that CA had generally not been able to improve the structural conditions compared to intensive tillage, yielding an increase in soil bulk density. They postulated that the limited amount of non-complexed organic carbon available for interaction with the clay minerals and the low ratio between clay and silt particles prevented a resilient soil structure even in soils treated with CA practices.

The aim of this study is to evaluate the effect of CA practices on the soil pore network in terms of total porosity, pore size distribution, morphology and architecture in the same silty soils as those studied by Piccoli et al. (2016b). Coupling mercury intrusion

porosimetry and x-ray micro tomography we investigated the porosity from the macro- to the ultramicro- scale on 96 samples collected in a field experiment set up on four farms in which CA practices (no-tillage, cover crops and residues retention) were applied and compared to conventional tillage system over a 5-yr transition period.

The hypothesis tested in this study is that CA could positively affect pore architecture and morphology, offsetting the effects of soil compaction. Greater organic matter content and biological activity, at least at shallow depth, could lead to higher macropore volume, connectivity and pore vertical orientation.

2 Materials and methods

2.1 Experimental sites

A field experiment was set up on four farms located in Veneto Region (North-eastern Italy) (Fig.1). Farm 1 (F1) was situated in a reclaimed area on the Adriatic coast ($45^{\circ} 38.350'N$ $12^{\circ} 57.245'E$, -2 m a.s.l.), the soil was Endogleyic Fluvisols (WRB, 2006) with a texture ranging from silty clay loam to silt loam (Table 1). The parent materials were calcareous silt sediments from the rivers Tagliamento and Piave. Farm 2 (F2) was located on an ancient low-lying plain originated from calcareous silt deposits of the River Brenta ($45^{\circ} 34.965'N$ $12^{\circ} 18.464'E$, 6 m a.s.l.) and had Endogleyic Calcisols (WRB, 2006) with a silty clay loam/silt loam texture. Farm 3 (F3) was located on a recent low-lying plain on the edge of the Venice lagoon ($45^{\circ}22'48.62''N$ $12^{\circ} 9'47.84''E$, 1 m a.s.l.) with Haplic Cambisols soils (WRB, 2006). The loamy texture was originated from calcareous deposits of the rivers Brenta and Bacchiglione. Farm 4 (F4) was located further to the west on the recent southern low-lying plain of the River Po ($45^{\circ} 2.908'N$ $11^{\circ} 52.872'E$, 2 m a.s.l.). Its soils were Gleyic Phaeozems (WRB, 2006) with a silty clay loam or silt loam texture.

The climate in the region was subhumid with annual rainfall around 829 mm in F1, 846 mm in F2, 859 mm in F3 and 673 mm in F4. In the median year, rainfall was highest in autumn (302, 241, 246 and 187 mm for F1, F2, F3 and F4, respectively) and lowest in winter (190, 157, 170 and 129 mm). Temperatures increased from January (minimum average: -0.1, -0.9, -0.8 and -0.2 °C respectively) to July (maximum average: 29.6, 29.3, 29.5 and 30.6 °C respectively). Reference evapotranspiration (ET₀) was 860, 816, 792

and 848 mm, with a peak in July (4.9, 4.6, 4.6 and 4.8 mm d⁻¹). ETo exceeded rainfall from May to September in F1, F2 and F3 and from May to October in F4.

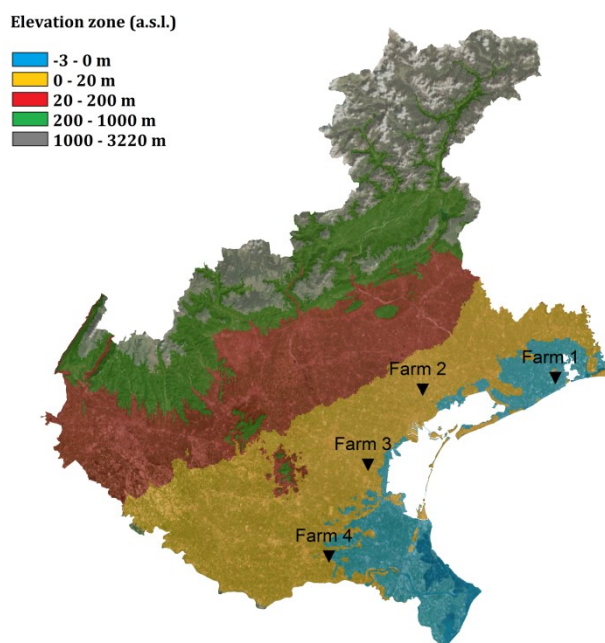


Figure 1 - Experimental sites in Veneto Region low plain, north-eastern Italy. Farms positions are marked with triangles.

Table 3 - Soil textural composition and organic carbon of experimental farms.

Farm	Layer	Sand (50-200 μm) g 100 g ⁻¹	Silt (2-50 μm) g 100 g ⁻¹	Clay (< 2 μm) g 100 g ⁻¹	Fine20 (< 20 μm) g 100 g ⁻¹	SOC g 100 g ⁻¹
F1	L1	19.1	49.9	31.0	58.5	1.7
	L2	13.6	53.2	33.2	62.1	1.3
	L3	15.5	52.7	31.9	61.1	1.3
	L4	18.2	51.0	30.8	57.1	1.1
F2	L1	11.5	61.3	27.2	69.1	1.3
	L2	12.6	60.9	26.5	71.6	1.0
	L3	10.9	61.4	27.6	72.3	1.0
	L4	9.3	63.6	27.1	74.6	0.9
F3	L1	50.0	36.8	13.2	33.5	0.8
	L2	47.1	39.1	13.9	35.5	0.6
	L3	47.2	38.9	13.9	35.4	0.6
	L4	50.2	36.0	13.8	33.3	0.5
F4	L1	18.9	51.5	29.6	62.9	1.4
	L2	15.7	54.9	29.5	65.8	1.3
	L3	16.3	53.8	29.9	65.3	1.1
	L4	15.7	52.7	31.6	66.1	1.0

F1: farm 1; F2: farm 2; F3: farm 3; F4: farm 4; L1: 3-5.5; L2: 12-14.5 cm; L3: 20-22.5 cm;
L4: 45-47.5 cm

2.2 The experiment

Experimental treatments were established at each farm in 2010 in order to compare conventional “intensive tillage” (IT) and conservation agriculture (CA). Experimental fields were rectangular (about 400 m length x 30 m width) with an average size of 1.2 ha. IT consisted of traditional tillage practices based on mouldboard ploughing (35 cm) with crop residues incorporation followed by secondary tillage (i.e. disk harrowing) while CA included sod seeding (direct drilling), residues retention on the soil surface and use of cover crops. The crop rotation (four-year) was the same in both treatments: wheat (*Triticum aestivum* L.), oilseed rape (*Brassica napus* L.), maize (*Zea mays* L.) and soybean (*Glycine max* (L.) Merr.). From 2014 a simplified three-year crop rotation wheat-maize-soybean was applied. In CA, cover crops were also grown between the main crops: sorghum (*Sorghum vulgare* Pers. var. *sudanense*) during spring-summer, with a mixture of vetch (*Vicia sativa* L.) and barley (*Hordeum vulgare* L.) until 2014 and then only barley or winter wheat during autumn-winter. The soil instead remained bare between the main crops in the IT treatment.

In IT, base dressing fertilizer was applied 1-2 weeks before sowing while subsurface band fertilization was applied at sowing in CA. In both management systems mineral fertilization was integrated by side dressing in maize (1 treatment) and wheat (2 treatments). There was no additional fertilization for cover crops while pesticide applications depended on crop requirements and were the same for both treatments. Before spring sowing, N-(phosphonomethyl) glycine was applied to suppress the winter cover crop in CA while sorghum suppression was achieved mechanically, through shredding.

2.3 Soil sampling

Soil sampling was done, contemporary in all four farms, in July 2015 in the inter-row at soybean full bloom. In each treatment, three undisturbed soil cores (7 cm diameter) down to 50 cm were collected through a hydraulic sampler at the same progressively increased distance from the field edge (5 m, 50 m and 150 m). Disturbed soil samples were also collected in the same position for particle size distribution and soil organic carbon analyses. Soil cores were then cut in 4 layers: 3-5.5 cm (L1), 12-14.5 (L2), 20-22.5 (L3) and 45-47.5 cm (L4).

Due to the technical constraints of x-ray microtomography scan, soil core size was reduced by carefully pulling a cylindrical plastic ring (1.7 cm × 2.5 cm) through the moist sample to avoid soil compaction. Soil cores and bulk samples were stored at 4 °C until analyses.

2.4 Particle size distribution

Particle size distribution was determined on disturbed soil samples after being air-dried, sieved at 2 mm and shaken for 12 h at 80 rpm in 2% sodium hexametaphosphate solution. Particle size distribution was determined through laser diffraction method (Mastersizer 2000, Malvern Instruments) and a dedicated algorithm was used to convert diffraction values into pipette ones. Fine20 was then calculated as the fraction of particles less than 20 µm.

2.5 Soil organic carbon

Before soil organic carbon (SOC) determination air-dried soil samples were crushed by rolling pin to break up clods and pass through a 0.5 mm sieve. SOC was determined by dichromate oxidation (Walkley & Black, 1934). The Hassink ratio was then calculated as the ratio between Fine20 and SOC.

2.6 Soil porosity

Soil porosity was analysed from micro- to millimetre scale coupling mercury intrusion porosimetry (0.0074-100 μm) and x-ray computed microtomography ($>26 \mu\text{m}$).

2.6.1 X-ray computed microtomography

X-ray computed microtomography (x-ray μCT) was performed on the undisturbed soil cores using a Skyscan 1172 (Bruker MicroCT, Belgium) at the University of Padova facility. X-ray source was set at 100kV and 100 μA . Projections were collected during a 180° sample rotation at 0.3° angular incremental step, each projection was the average of 9 frames collected with an exposure time of 1500 msec. Beam hardening effect was minimized during the acquisition using a 0.5 mm aluminium filter, pixel size was 26 μm .

8-bit images (slices) were then reconstructed from 16-bit projections using NRecon software (Bruker MicroCT, Belgium). Reconstruction parameters involved a 2 pixels smoothing to reduce noise, adequate misalignment compensation and ring artefacts reduction. The final image stack was of 635 slices with 26 μm thickness per slice and a 26 μm resolution. The image analysis was conducted with CTAn software (Bruker MicroCT, Belgium). The soil cores dataset was digitally cropped to a region of interest (ROI) 1.5 cm in diameter corresponding to the maximum inscribed cylinder for all soil core stacks. Thresholding was determined by Otsu's method (Otsu, 1979) and all objects <5 pixels were removed since affected by systematic error (Vogel *et al.*, 2010). Total porosity (TP_ μCT) was calculated dividing the pore voxels number by the total volume voxels number. Pore size distribution (PSD) was obtained as local thickness as defined by Hildebrand and Ruesegger (1997): for a point in a solid represents the diameter of the largest sphere that encloses the point and is entirely bounded within the solid surface. The process first involves skeletonisation along the medial axes and then the measurement of the sphere-fitting local thickness for all the voxels lying along the axis. The computation output is a thickness histogram with an interval of two pixels. In order to facilitate statistical comparison between treatments, pores were classified into five

classes: 26-500 (CL1_μCT), 500-1000 (CL2_μCT), 1000-1500 (CL3_μCT), 1500-2000 (CL4_μCT) and >2000 μm (CL5_μCT). Degree of anisotropy (DA_μCT) was calculated from the concepts of mean intercept length (MIL) (Harrigan & Mann, 1984) and Eigen analysis; it is an indicator of the 3D pores symmetry and its values range from 0 (symmetrical structure) to 1 (anisotropic structure). A volume can be defined anisotropic if there is a preferential alignment along a directional axis and a line passing through the volume in any direction will make a different number of intercepts through the object. Fractal dimension (FD_μCT) describes the self-similarity (scale invariance) of an object, quantifying how its surface fills the space, and is an indicator of surface complexity. FD_μCT was calculated by the Kolmogorov method (“box counting”) which, for 3D calculation, implies total volume divided by an array of equal cubes, and the number of cubes containing the object surface is counted. This process is repeated over a cube length range 2-100 pixels. FD_μCT is then calculated as the slope of log-log regression between number of voxels and cube length. The connectivity density (CD_μCT) was computed through the following equation:

$$CD_{\mu CT} = \frac{\beta_0 - \beta_1 + \beta_2}{VOI} \quad [1]$$

where the numerator represents the Euler-Poincare formula for a 3D object (β_0 : pores number, β_1 : connectivity and β_2 : number of enclosed cavities) and VOI is the analysed volume of interest (μm^3). Pore orientation (or_μCT) was defined as the orientation (in degrees) of the pore major axis of the object and was defined in the upper hemisphere only with values from 0-90°. Pore sphericity (sph_μCT) was calculated as the ratio of the surface area of a sphere (with the same volume - V - as the given particle) to the surface (S) area of the particle:

$$Sph_{\mu CT} = \frac{\sqrt[3]{\pi(6V)^{2/3}}}{S} \quad [2]$$

For a complex, non-spherical object the surface area of the volume-equivalent sphere will be much smaller than the particle surface area, thus sphericity will be low. The maximum value possible is 1, which would be obtained for a perfect sphere.

After the x-ray μCT scan and plastic cylinder carefully removal, the samples were oven-dried at 105 °C for 24 h to calculate total porosity (TP_core) from soil bulk density (BD) (core method, Grossman and Reinsch, 2002) and particle density (PD) (helium

pycnometer, Micro Ultrapyc 1200e, Quantachrome, England). Samples were then subjected to mercury intrusion porosimetry.

2.6.2 Mercury intrusion porosimetry

The samples were analysed by mercury intrusion porosimetry (MIP) to investigate the total accessible porosity (TP_MIP) and the PSD in the 0.0074 μm - 100 μm range. Thermo Finningan (Waltman, USA) Pascal 140 and 240 were used to investigate the 3.8-100 μm and 0.0074-15 μm porosity ranges respectively. The pore radius of the mercury (Hg) intrusion was calculated with the Young-Laplace equation as a function of pressure (P) and being dependent on Hg surface tension (γ) (0.48 N m^{-1}) and Hg-soil contact angle (θ) (140°):

$$r = \frac{2\gamma \cos \theta}{P} \quad [3]$$

As suggested by Cameron and Buchan (2006), pore classes were defined as: cryptopores (crypto_MIP) (0.0074-0.1 μm), ultramicropores (ultramicro_MIP) (0.1-5 μm), micropores (micro_MIP) (5-30 μm), mesopores (meso_MIP) (30-75 μm) and macropores (macro_MIP) (75-100 μm).

Fractal dimension (FD_MIP) index was calculated as:

$$FD_MIP = Sl - 4 \quad [4]$$

where Sl is the slope of the porosity function where volume-pressure ratio (log scale) is plotted against pressure (log scale) (Friesen and Laidlaw, 1993).

Tortuosity (T_MIP) was derived according to Carniglia (1986):

$$T_MIP = 2.23 - 1.13 V_{max} BD \quad [5]$$

where V_{max} is the maximum pore volume and BD the bulk density.

2.7 Statistical analysis

Statistical analysis was applied in order 1) to test the effect of treatment, soil layer and soil characteristics (soil organic carbon, sand and fine20 contents) through a mixed-effect model applied to all studied *i-th* variables as follow:

$$\text{Variable}_i = \text{SOC} + \text{Sand} + \text{Fine20} + \text{Treatment} + \text{Layer} + \text{Farm} + \text{Treatment} \times \text{Layer} + \text{Treatment} \times \text{Farm} \quad [6]$$

where *SOC*, *sand* and *Fine20* represented the continuous factors while *layer*, *treatment*, *farm*, *treatment* × *layer* and *treatment* × *farm* the categorical ones. *Farm* effect was considered as random effect, while all the others as fixed. Post-hoc pairwise comparisons of least-squares means (LSE) were performed, using the Tukey method to adjust for multiple comparisons; 2) to estimate all possible linear relationships between analysed variables: Pearson's correlation coefficient was calculated and reported in the correlation matrix; 3) to highlight the general interdependence between pore size distribution, morphology, soil organic carbon and texture-derived indices, a principal component analysis (PCA) on 11 selected variables was adopted. The variables were selected according to Kaiser's measure of sampling adequacy (MSA), which resulted as 0.77 indicating that the group of variables was appropriate for the analysis (Kaiser, 1974). Only rotated orthogonal components with eigenvalues > 1 were extracted.

Statistical analyses were performed with SAS software (SAS Institute Inc. Cary, NC, USA), 5.1 version.

3 Results

3.1 Soil porosity

3.1.1 Total porosity

Total porosity (TP) was calculated by mercury intrusion porosimetry (TP_MIP) in the pore range 0.0074 μm-100 μm, by x-ray μCT (TP_μCT) for pores > 26 μm and according to bulk density (TP_core). TP was 4.92E-02 (IT) vs 4.52E-02 (CA), 3.00E-01 (IT) vs

3.09E-01 (CA) and 3.28E-01 (IT) vs 3.31E-01 (CA) $\mu\text{m}^3 \mu\text{m}^{-3}$ for x-ray μCT , MIP and core method respectively (Table 2) without significant effect of treatment (Table 3). Regardless of the adopted method, TP significantly decreased with depth ($p \leq 0.05$) (Table 3, Fig.2) and despite an overlapping range (26-100 μm) between MIP and x-ray μCT , it can be noticed that majority of porosity (about 90%) was $< 100 \mu\text{m}$ as measured by MIP (Fig.2).

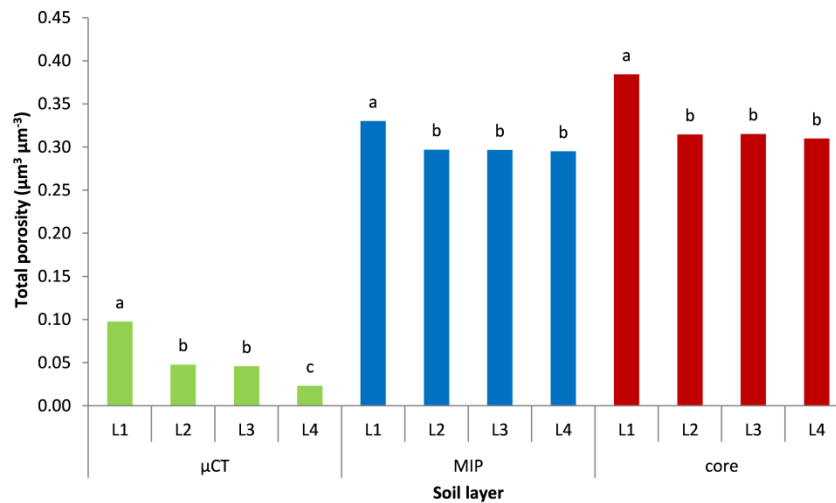


Figure 2 - Distribution of total porosity according to the three used methods as affected by soil depth. Different letters indicate significant differences (Tukey post-hoc test with $p \leq 0.05$). L1: 3-5.5 cm; L2: 12-14.5 cm; L3: 20-22.5 cm; L4: 45-47.5 cm; μCT : x-ray computed microtomography; MIP: mercury intrusion porosimetry; core: measurement from bulk density.

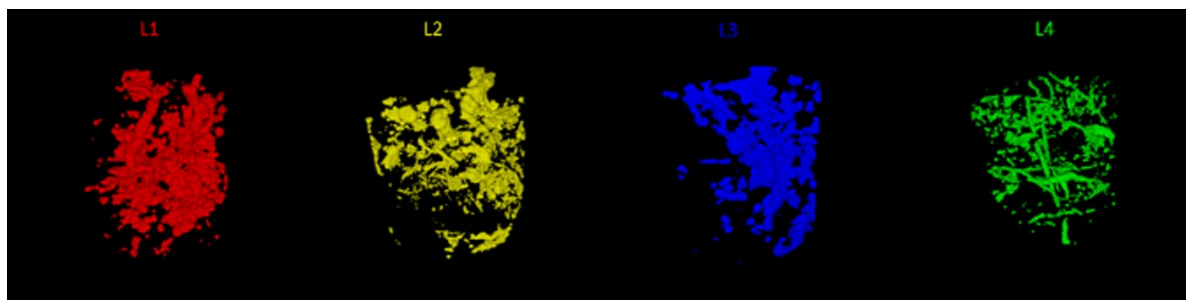


Figure 3 - Total porosity (μCT -derived) decreasing according to the soil depth. L1: 3-5.5 cm; L2: 12-14.5 cm; L3: 20-22.5 cm; L4: 45-47.5 cm.

Table 2 - Average values followed by standard error of μ CT-derived, MIP-derived and core-derived soil pore parameters in intensive tillage (IT) and conservation agriculture (CA). Pore parameters differ significantly between treatments when labelled with different letters (Tukey post-hoc test with $p \leq 0.05$).

Parameter	Unit	IT	CA
μ CT-derived			
TP_ μ CT	$\mu\text{m}^3 \mu\text{m}^{-3}$	4.92E-02 \pm 7.55E-03	4.52E-02 \pm 7.63E-03
FD_ μ CT	-	2.20 \pm 0.04	2.15 \pm 0.03
CD_ μ CT	μm^{-3}	9.29E-10 \pm 1.35E-10	8.85E-10 \pm 1.20E-10
DA_ μ CT	-	0.27 \pm 0.02	0.27 \pm 0.01
Or_ μ CT	$^\circ$	72.12 \pm 0.86	75.94 \pm 0.76
Sph_ μ CT	μm	7.80E-01 \pm 1.78E-03	7.84E-01 \pm 1.50E-03
CL1_ μ CT	$\mu\text{m}^3 \mu\text{m}^{-3}$	4.15E-02 \pm 4.98E-03	3.48E-02 \pm 3.78E-03
CL2_ μ CT	$\mu\text{m}^3 \mu\text{m}^{-3}$	1.18E-02 \pm 2.14E-03	1.04E-02 \pm 2.50E-03
CL3_ μ CT	$\mu\text{m}^3 \mu\text{m}^{-3}$	2.81E-03 \pm 1.19E-03	3.90E-03 \pm 1.24E-03
CL4_ μ CT	$\mu\text{m}^3 \mu\text{m}^{-3}$	4.30E-04 \pm 4.22E-04	4.85E-04 \pm 5.92E-04
CL5_ μ CT	$\mu\text{m}^3 \mu\text{m}^{-3}$	6.19E-05 \pm 2.16E-04	1.42E-04 \pm 5.89E-04
MIP-derived			
TP_MIP	$\mu\text{m}^3 \mu\text{m}^{-3}$	3.00E-01 \pm 1.13E-02	3.09E-01 \pm 8.06E-03
FD_MIP	-	2.91 \pm 0.03	2.93 \pm 0.02
T_MIP	-	1.88 \pm 0.01	1.88 \pm 0.01
Macro_MIP	$\mu\text{m}^3 \mu\text{m}^{-3}$	6.81E-03 \pm 9.98E-04	5.91E-03 \pm 7.24E-04
Meso_MIP	$\mu\text{m}^3 \mu\text{m}^{-3}$	8.17E-02 \pm 4.08E-03	6.74E-02 \pm 2.18E-03
Micro_MIP	$\mu\text{m}^3 \mu\text{m}^{-3}$	1.52E-01 \pm 8.72E-03	1.43E-01 \pm 7.40E-03
Ultramicro_MIP	$\mu\text{m}^3 \mu\text{m}^{-3}$	1.67E-01 \pm 8.08E-03	1.86E-01 \pm 5.78E-03
Cripto_MIP	$\mu\text{m}^3 \mu\text{m}^{-3}$	3.68E-02 \pm 3.04E-03	3.85E-02 \pm 2.90E-03
core-derived			
TP_core	$\mu\text{m}^3 \mu\text{m}^{-3}$	3.28E-01 \pm 1.16E-02	3.31E-01 \pm 9.13E-03

TP_ μ CT: total porosity (μ CT-derived); FD_ μ CT: fractal dimension (μ CT-derived); CD_ μ CT: connectivity density; DA_ μ CT: degree of anisotropy; Or_ μ CT: orientation; Sph_ μ CT: sphericity; CL1_ μ CT: 26-500 μm ; CL2_ μ CT: 500-1000 μm ; CL3_ μ CT: 1000-1500 μm ; CL4_ μ CT: 1500-2000 μm ; CL5_ μ CT: >2000 μm ; TP_MIP: total porosity (MIP-derived); FD_MIP: fractal dimension (MIP-derived); T_MIP: tortuosity; Macro_MIP: 75-100 μm ; Meso_MIP: 30-75 μm ; Micro_MIP: 5-30 μm ; Ultramicro_MIP: 0.1-5 μm ; Crypto: 0.0074-0.1 μm ; TP_core: total porosity (core method).

Table 3 - Comparison of significance level among the linear mixed-effect model analysis of total porosity MIP-derived (TP_MIP), μ CT-derived (TP_ μ CT) and from bulk density (TP_core), cryptopores (crypto_MIP), ultramicropores (ultramicro_MIP), micropores (micro_MIP), mesopores (meso_MIP), macropores (meso_MIP), 26-500 porosity class (CL1_ μ CT), 500-1000 μ m porosity class (CL2_ μ CT), 1000-1500 μ m porosity class (CL3_ μ CT), 1500-2000 μ m porosity class (CL4_ μ CT), >2000 μ m porosity class (CL5_ μ CT), tortuosity (T_MIP), fractal dimension MIP-derived (FD_MIP), pores orientation (or_ μ CT), fractal dimension μ CT-derived (FD_ μ CT), connectivity density (CD_ μ CT), pore sphericity (sph_ μ CT) and degree of anisotropy (DA_ μ CT).

Variable	SO	San	Fine2	Treatmen	Laye	Treatment×LayerNu	Treatment×Far
TP_MIP	n.s.	n.s.	n.s.	n.s.	0.05	n.s.	<0.01
TP_ μ CT	n.s.	n.s.	n.s.	n.s.	<0.01	n.s.	0.02
TP_core	n.s.	n.s.	n.s.	n.s.	0.01	n.s.	n.s.
Crypto_MIP	n.s.	n.s.	<0.01	n.s.	0.01	n.s.	n.s.
Ultramicro_MI	n.s.	n.s.	n.s.	<0.01	n.s.	n.s.	n.s.
Micro_MIP	n.s.	0.01	<0.01	n.s.	n.s.	n.s.	n.s.
Meso_MIP	n.s.	0.02	n.s.	0.04	<0.01	n.s.	n.s.
Macro_MIP	n.s.	n.s.	n.s.	n.s.	<0.01	n.s.	n.s.
CL1_ μ CT	n.s.	n.s.	n.s.	n.s.	<0.01	n.s.	n.s.
CL2_ μ CT	n.s.	n.s.	n.s.	n.s.	<0.01	n.s.	n.s.
CL3_ μ CT	n.s.	n.s.	n.s.	n.s.	<0.01	n.s.	n.s.
CL4_ μ CT	n.s.	n.s.	n.s.	n.s.	0.03	n.s.	n.s.
CL5_ μ CT	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
T_MIP	n.s.	n.s.	0.05	n.s.	0.01	n.s.	n.s.
FD_MIP	n.s.	n.s.	<0.01	n.s.	n.s.	n.s.	<0.01
Or_ μ CT	n.s.	0.02	0.02	<0.01	n.s.	n.s.	<0.01
FD_ μ CT	n.s.	n.s.	n.s.	n.s.	<0.01	n.s.	n.s.
CD_ μ CT	n.s.	n.s.	n.s.	n.s.	<0.01	n.s.	n.s.
Sph_ μ CT	n.s.	0.05	0.03	n.s.	n.s.	n.s.	<0.01
DA_ μ CT	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

SOC: soil organic carbon; Fine20: particles < 20 μ m.

3.1.2 Pore size distribution

Pore size distribution (PSD) investigated by MIP was influenced by treatment and layer (Table 3). CA increased the ultramicroporosity ($1.86\text{E-}01$ vs $1.67\text{E-}01 \mu\text{m}^3 \mu\text{m}^{-3}$, Table 2) as a result of a decrease in the mesoporosity fraction ($6.74\text{E-}02$ vs $8.17\text{E-}02 \mu\text{m}^3 \mu\text{m}^{-3}$, Table 2). Irrespective of treatment, macro- and mesoporosity decreased with depth, contrary to criptoporosity that showed high values in the deepest layer ($p \leq 0.01$) (Table 4). MIP porosity classes also varied according to the texture properties, sand and fine20 being positively correlated with meso- and criptopores and negatively with micropores (Table 3). Generally ultramicro- and micro- followed by mesoporosity class dominated the $0.0074\text{-}100 \mu\text{m}$ range (90.2% in IT vs 89.9% in CA), while the macroporosity fraction was negligible (Table 2 and 4).

PSD by x-ray μCT ($>26 \mu\text{m}$) did not show any significant treatment-related effect (Table 3), on average $4.15\text{E-}02$ vs $3.48\text{E-}02 \mu\text{m}^3 \mu\text{m}^{-3}$ in CL1, $1.18\text{E-}02$ vs $1.04\text{E-}02 \mu\text{m}^3 \mu\text{m}^{-3}$ in CL2, $2.81\text{E-}03$ vs $3.90\text{E-}03 \mu\text{m}^3 \mu\text{m}^{-3}$ in CL3, $4.30\text{E-}04$ vs $4.85\text{E-}04 \mu\text{m}^3 \mu\text{m}^{-3}$ in CL4 and $6.19\text{E-}05$ vs $1.42\text{E-}04 \mu\text{m}^3 \mu\text{m}^{-3}$ in CL5 for IT and CA respectively (Table 2, Fig.3). μCT -derived PSD showed instead significant differences according to the layer (Table 3), with a progressive decrease of CL1-CL4 according to the depth while CL5 did not show any variation (Table 4). The μCT -derived porosity was dominated by CL1 (26-500 μm) followed by CL2 (500-1000 μm) and CL3 (1000-1500 μm), whereas classes $> 1500 \mu\text{m}$ represented only ca. 1% of the macroporosity (Table 4, Fig.4).

Table 4 - Pore size distribution ($\mu\text{m}^3 \mu\text{m}^{-3}$) of different soil layers examined with mercury intrusion porosimetry and x-ray μCT . Pore classes differ significantly along the soil depth when labelled with different letters (Tukey post-hoc test with $p \leq 0.05$).

Pore size class Name	Range (μm)	Soil layer							
		3-5.5 cm	12-14.5 cm	20-22.5 cm	45-47.5 cm				
Crypto_MIP	0.0074-0.1	3.52E-02	b	3.66E-02	b	3.54E-02	b	4.38E-02	a
Ultramicro_MIP	0.1-5	1.75E-01		1.81E-01		1.82E-01		1.67E-01	
Micro_MIP	5-30	1.62E-01		1.34E-01		1.36E-01		1.59E-01	
Meso_MIP	30-75	1.14E-01	a	6.53E-02	b	6.29E-02	b	6.10E-02	b
Macro_MIP	75-100	1.27E-02	a	4.89E-03	b	4.84E-03	b	4.42E-03	b
CL1_ μCT	26-500	7.00E-02	a	3.58E-02	b	3.17E-02	b	2.19E-02	b
CL2_ μCT	500-1000	2.38E-02	a	1.14E-02	b	9.64E-03	b	3.78E-03	c
CL3_ μCT	1000-1500	6.72E-03	a	4.28E-03	a	3.84E-03	a	4.57E-04	b
CL4_ μCT	1500-2000	5.56E-04	ab	4.40E-04	ab	1.24E-03	a	3.31E-05	b
CL5_ μCT	>2000	1.65E-05		2.15E-04		2.83E-04		1.59E-05	

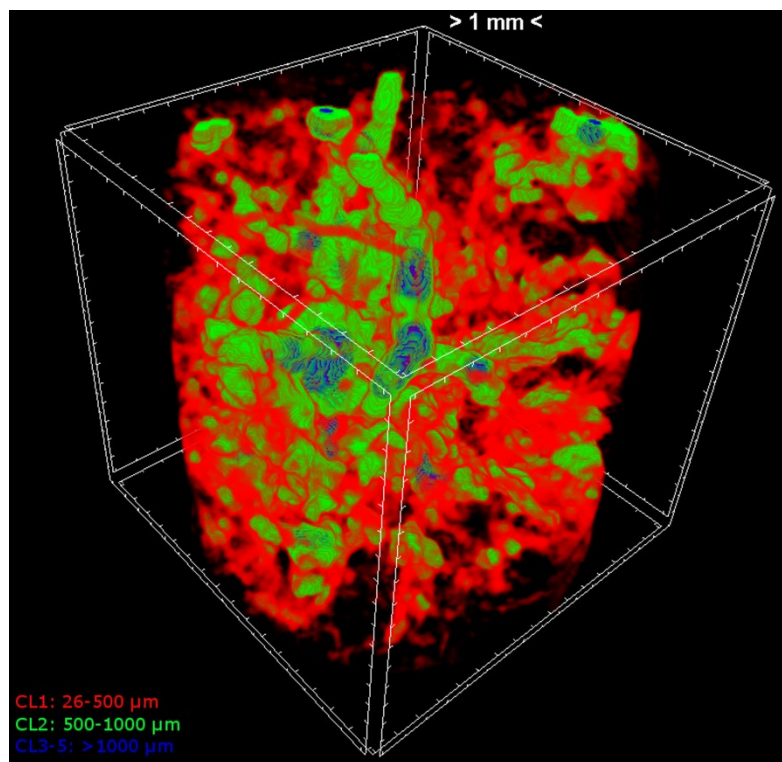


Figure 4 - Representation of 3D sphere-fitting from x-ray μCT -scan. 26-500 μm (CL1) (in red), 500-1000 μm (CL2) (in green) and >1000 μm classes (CL2-CL5).

3.1.3 Pore architecture and morphology

Pore architecture in the MIP range varied within the profile (Table 3), the tortuosity (T_{MIP}) being 1.85 in L1, 1.88 in L4 and 1.89 in L3 and L2 without a treatment effect. It also resulted as being affected by texture, with T_{MIP} and the fractal dimension (FD_{MIP}) positively correlated with fine20 (Table 3).

The treatment resulted as significant (Table 3) only for pore orientation measured by x-ray μ CT, the pore being more vertical in CA (76°) than IT (72°) (Table 2). Pore orientation was also positively influenced by the texture properties ($p=0.02$, Table 3). Fractal dimension calculated within the x-ray μ CT pore domain ($FD_{\mu CT}$) decreased significantly ($p<0.01$) with depth, the value being 2.4, 2.2, 2.1 and 2.0 for L1, L2, L3 and L4 respectively with no differences between treatments (2.20 IT vs 2.15 CA), whereas pore connectivity resulted as significantly higher (lower $CD_{\mu CT}$ index value) in deeper layers (7.54×10^{-10} as a mean) than in the upper one (L1) (1.64×10^{-9}). Finally, the degree of anisotropy ($DA_{\mu CT}$), showed a random pattern regardless of treatment, layer or texture properties (Table 3).

From a morphologic point of view, sphericity ($sph_{\mu CT}$) parameter discriminates treatment on F1, with CA pores slightly more spherical than IT (0.79 vs 0.78), while no differences were observed on the other farms. Sphericity was also positively influenced by sand and fine20 fractions (Table 3).

3.2 Relationships between MIP- and x-ray μ CT-derived parameters and soil chemical-physical characteristics

Soil organic carbon (SOC) was positively correlated ($p<0.05$) with cryptopores ($r=0.5$) and ultramicropores ($r=0.3$) in the MIP-domain and with 26-1500 μ m μ CT-classes ($r=0.3$), while negatively only with intermediate MIP-derived classes (micro- and mesopores, $r=0.6$ and 0.4 respectively) (Fig.5). MIP-derived classes were also strongly affected by tortuosity (T_{MIP}) (Fig.5), higher classes (macro-, meso-, micro- and ultramicropores) and lower class (criptopores) being negatively and positively correlated respectively. Fractal dimensions, estimated in two different domains (FD_{MIP} and $FD_{\mu CT}$), were weakly ($r=0.3$) but significantly negatively correlated (Fig.5), the former being

positively correlated with tortuosity ($r=0.7$) while the latter with connectivity density ($CD_{\mu CT}$) ($r=0.9$) (Fig.5). $CD_{\mu CT}$ was then negatively correlated with orientation ($or_{\mu CT}$) which was positively affected by pores sphericity ($sph_{\mu CT}$) ($r=0.8$) (Fig.5).

A general overview of factors influencing the soil structure was also provided by PCA (Fig.6, Table5). Two principal components were extracted that explained 42% (PC1) and 37% (PC2) of variance. The first principal component was associated with the microporosity domain being positively correlated with fine20, FD_{MIP} and criptopores and negatively with micropores (Table 5). The second one was representative of macropores resulting positively correlated with μCT -derived parameter ($TP_{\mu CT}$, $CL1_{\mu CT}$, $CL2_{\mu CT}$, and $FD_{\mu CT}$) and MIP macropores (Table 5). In the plane described by PC1 and PC2, PC1 separated F3 from the other three studied farms, the former being less associated with the microscale domain than the others. Moreover, within the same farm, PC2 discriminated the two treatments, CA being less correlated with the macroscale-related component (Fig.6). Lastly, PC2 separated the top layer (L1) from the deeper ones (L2-L4) (Fig.6) as it was more represented by macroporosity-related characteristics.

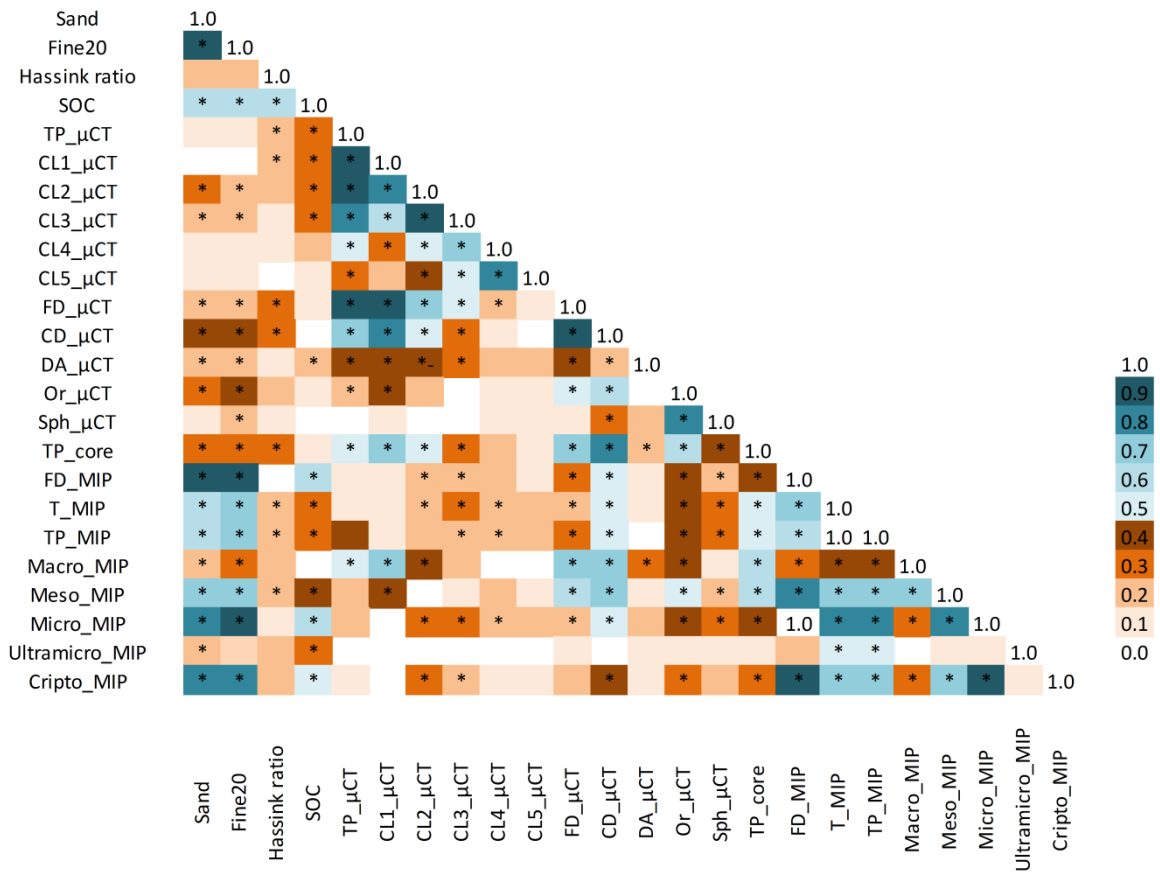


Figure 5 - Correlation matrix for analysed MIP- and μCT-derived parameters and chemical-physical properties. Fine20: particles less than 20 μm; Hassink ratio: fine20-SOC ratio; SOC: soil organic carbon; TP_μCT: total porosity (μCT-derived); CL1_μCT: 26-500 μm; CL2_μCT: 500-1000 μm; CL3_μCT: 1000-1500 μm; CL4_μCT: 1500-2000 μm; CL5_μCT: >2000 μm; FD_μCT: fractal dimension (μCT-derived); CD_μCT: connectivity density; DA_μCT: degree of anisotropy; Or_μCT: orientation; Sph_μCT: sphericity; TP_core: total porosity (core method); FD_MIP: fractal dimension (MIP-derived); T_MIP: tortuosity; Macro_MIP: 75-100 μm; Meso_MIP: 30-75 μm; Micro_MIP: 5-30 μm; Ultramicro_MIP: 0.1-5 μm; Crypto: 0.0074-0.1 μm.

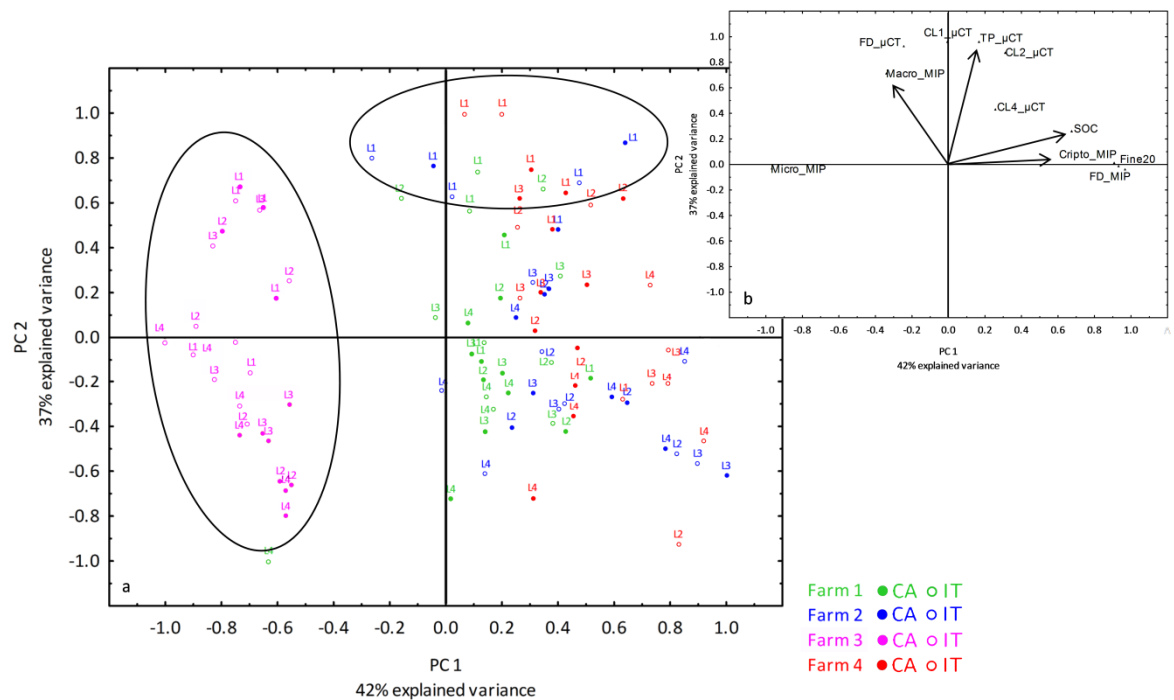


Figure 6 - Principal component analysis of selected variables. 4-a represents case scores where different colour of labels highlights different experimental farms (farm 1 in green, farm 2 in blue, farm 3 in pink and farm 4 in red). Different labels correspond to the studied soil layers; L1: 3-5.5 cm, L2: 12-14.5 cm, L3: 20-22.5 cm and L4: 45-47.5 cm. Black circles highlighted the PCA discriminated clusters. 4-b represents factor loadings: Macro_MIP: 75-100 μm , Micro_MIP: 5-30 μm , Crypto_MIP: 0.0074-0.1 μm , FD_MIP: fractal dimension (MIP-derived), Fine20: particles < 20 μm , SOC: soil organic carbon, CL1_μCT: 26-500 μm , CL2_μCT: 500-1000 μm , CL4_μCT: 1500-2000 μm and FD_μCT: fractal dimension (μCT -derived).

Table 5 - Correlation between extracted principal components (PC1 and PC2) and variables (bolded factors >0.7).

Variable	PC1	PC2
Fine20	0.93	-0.01
SOC	0.68	0.26
TP_μCT	0.17	0.97
CL1_μCT	-0.01	0.97
CL2_μCT	0.31	0.88
CL4_μCT	0.26	0.43
FD_μCT	-0.25	0.93
FD_MIP	0.97	-0.04
Macro_MIP	-0.34	0.71
Micro_MIP	-0.97	-0.03
Crypto_MIP	0.91	0.01
Eigenvalue	4.58	4.08
Explained variance	41.63	37.12

Fine20: particles < 20 μm; SOC: soil organic carbon; (TP_μCT): total porosity > 26 μm;
 CL1_μCT: 26-500 μm porosity class; CL2_μCT: 500-1000 μm porosity class;
 CL4_μCT: 1500-2000 μm porosity class; FD_MIP: fractal dimension MIP-derived;
 macro_MIP: 75-100 μm porosity class; micro_MIP: 5-30 μm porosity class;
 crypto_MIP: 0.0074-0.1 μm porosity class.

4 Discussion

Understanding the soil structure at microscopic level is particularly appropriate for fine-textured soils as it can be helpful for explaining and modelling higher structural level behaviours under different hydro-mechanical stress conditions (Romero & Simms, 2008). On the contrary, macropore networks analyses provide insights into the water and air dynamics as affected by soil management (Iversen *et al.*, 2012; Katuwal *et al.*, 2015).

Porosity distribution in silty soils of Veneto plain was primarily in the microporosity range (0.0074 μm -30 μm) contributing to ca. 82% of total porosity, confirming the evidence already reported for similar soils by Dal Ferro *et al.* (2012). This behaviour was related not only to texture properties but also to the soils' compacting tendency. This is clearly confirmed by the positive relationship existing between total porosity (TP_core) calculated from bulk density (BD) and the macroporosity measured by x-ray μCT and MIP. Indeed, lower TP_core values caused by soil compaction were strongly associated to a contraction in meso- and macropore classes. Instead, criptopores (<0.1 μm) were texture-driven and related to the inner particle porosity (Cameron & Buchan, 2006).

The effects of soil management on pore size distribution (PSD) varied according to the pore range, PSD being affected in the MIP-domain (0.1-5 μm) but not in the x-ray μCT one (> 26 μm). These results are in accordance with Pagliai *et al.* (2004) who showed a positive influence of conservation tillage practices on the 0.5-50 μm porosity class. On the contrary other Authors (e.g. Dal Ferro *et al.*, 2014; Lipiec *et al.*, 2006) did not find any difference in the microporosity range between CA and conventional tillage. Kay and VandenBygaart (2002) related pore characteristics changes after no-tillage adoption to three main effects. Firstly, in the short term (seconds-days-months), soil compaction and fragmentation would be expected as a result of tillage absence and traffic; then, in the medium term (months-years), greater biological activity (e.g. higher amounts of earthworms) would alleviate soil strength through vertical-oriented bio-macropores; finally, after longer periods (years-decades), the different distribution of soil organic carbon (SOC) would be the key factor in soil structure stabilization.

The SOC-PSD relationship is complex and bidirectional since, on the one hand, different forms of organic matter stabilize different pore sizes (Kay & VandenBygaart, 2002) and, on the other, PSD can selectively protect specific carbon pools in different aggregate

size fractions as a result of pore space inaccessibility to microorganisms and enzymes (Lützow *et al.*, 2006). As an example, SOC protection from decomposition is enhanced in small microaggregates that are rich in pores $<0.2 \mu\text{m}$ diameter, which is considered to be the limiting size for bacteria access (Cameron & Buchan, 2006; Lützow *et al.*, 2006). In agreement with those findings, a dual effect was confirmed in this experiment as SOC favoured the formation of pores $< 5 \mu\text{m}$ (ultramicro- and cryptopores) in the MIP-domain and 26-1500 μm in the μCT one. SOC-PSD protection is also improved by clay minerals by intercalation or adsorption processes in micro- and cryptopores (Lützow *et al.*, 2006), which were strongly correlated with SOC in other studies on Veneto soils (Lugato *et al.*, 2009; Simonetti *et al.*, 2016). The same authors concluded that although the chemical-physical-biological complexity of processes and interactions govern SOC dynamics, there was evidence that soil porosity distribution could be a valuable indicator of the soil organic carbon sequestration capacity. Therefore the increased ultramicroporosity favoured by CA practices in Veneto silty soils would be expected to slow down C turnover allowing SOC accumulation even if in more labile forms (Lugato *et al.*, 2009).

A higher bio-macropore fraction in the CA system, as a result of greater biological activity (e.g. earthworms and roots) and the absence of tillage-related soil structure loosening was not observed in this study. Most likely the small volume of the soil cores limited the detection of very large pores. The importance of biopores has recently been emphasized by Naveed *et al.* (2016) as they sustain both soil and water quality through structure stabilization and water infiltration and drainage improvements. Indeed vertical oriented bio-macropores are resistant under traffic load (Blackwell *et al.*, 1990) and would be expected to extend deeper than in a previously tilled zone, so the benefits associated with biopores could involve the entire soil profile and be essential for no-tillage system on poorly drained and aerated soils. Although a macropore increase was not detectable in the CA treatment, from a morphological point of view the observed higher pore vertical orientation could be seen as a proxy for greater biological activity in CA management that could guarantee better soil drainage and aeration at small scale.

Despite the farms were located in different parts of Veneto Region, their soils showed similar characteristics. In particular soils of F1, F2 and F4 have similar texture, Fines20

and soil organic carbon contents, conversely F3 soil has a coarser properties. In spite of these different properties, no differences were observed between F3 and other farms in terms of pore size (i.e. pore size distribution), shape (i.e. pore morphology) or architecture (i.e. fractal dimension, connectivity density and degree of anisotropy). Only the PCA analysis allowed to identify the dependency of the macroscale domain from the coarser texture (F3).

The absence of a consistent soil structure improvement under CA management could be explained as related to the high silt content and the low soil organic matter contents which characterized the studied farms. Indeed Soane et al. (2012) reviewed the western and south-western European adoption of no-tillage confirming that soils with low structural stability and poor drainage are usually not suitable for no-tillage because of the higher compaction risk (Ehlers & Claupein, 1994; Munkholm *et al.*, 2013; Van Ouwerkerk & Perdok, 1994).

A higher soil compaction was usually reported in CA systems when tillage/no-tillage comparisons were < 10-yr (Kay & VandenBygaart, 2002; Palm *et al.*, 2014; Schjøning & Rasmussen, 2000) and was also confirmed by Piccoli et al. (2016a) for the same soils of Veneto plain. The Authors hypothesized that the high traffic load and absence of tillage operations during a 3-yr transition period were factors causing soil compaction. In this study, the bulk density was higher (average 1.8 g cm^{-3}) than previously measured by Piccoli et al. (2016a; 2016b) irrespective of farm or treatment. These results are scale-dependent since the two studies referred to different soil sample sizes (Baveye *et al.*, 2002).

Recently, Piccoli et al. (2016b) measured the degree of compactness (Keller & Håkansson, 2010) of 100 cm^3 cores collected from the same experiment. They demonstrated that Veneto low-lying silty soils are prone to compaction irrespective of agronomic management adopted due to the limited amount of non-complexed organic carbon available for interaction with clay minerals and the low clay-silt ratio.

5 Conclusions

The silty soils of Veneto plain showed a slow reaction to conservation agriculture adoption as demonstrated by the poor effect of conservation practices on the macropore networks. After 5-yr of CA vs IT comparison, few differences were recorded between treatments both in terms of pore size distribution, morphology or architecture. However the potential negative impact of soil compaction on porosity was most likely offset by the permanent soil covering with crop residues and cover-crops, which provided a consistent input of soil organic carbon.

The positive response of the ultramicropore (+11%) fraction to conservation practices indicates that a virtuous cycle was initiated between SOC and porosity during the transition period, hopefully leading to stabilized soil C pools, well-developed macropore systems and in turn enhanced soil functions and ecosystem services. However the chemical-physical properties of the silty soils (i.e. low structural stability and poor drainage) suggest that a longer transition phase will be required to reach a favourable equilibrium in the CA systems of the Veneto plain.

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Chapter V

General conclusions

Silty soils of the Veneto region low-lying plain showed a slow reaction to conservation agriculture (CA) practices, as demonstrated by the poor effect on C sequestration, gas-transport characteristics and soil structure improvements.

Soils experienced a general compaction that negated the exploitation of CA-related benefits. Soil structure became dense and dominated by the microporosity, resulting in poor gas exchanges leading to critical aeration and microbial aerobic activity.

This was in contrast with the initial hypotheses that identified fine and sorted soils as resilient to mechanical impact due to their natural limited inherent tendency to form dense layers. The high fraction of particles less than 20 μm , which is predominant in Veneto soils, should theoretically be able to complex-bind a large quantity of soil organic carbon (SOC) allowing structure stabilization. Therefore as a consequence of low SOC stock, the limited amount of non-complexed organic carbon available for interaction with the soil fines prevented the formation of a more resilient soil structure leading to soil compaction.

Despite such mechanisms, CA practices caused a SOC stratification within the profile concentrating the SOC in the topsoil and boosting a better humification process with the production of more polycondensed humic substances. C improvements together with the positive response of ultramicroporosity to CA practices suggest that a virtuous cycle between SOC and soil structure has been initiated, leading in the long term to soil C pools stabilization, well-developed macropore systems and in turn enhancing soil functions (e.g. aeration and drainage) and related ecosystem services.

In conclusion, the chemical-physical properties of the silty soils (i.e. low structural stability and poor drainage) indicate that a longer transition period will be needed to reach a favourable equilibrium in the CA systems of the Veneto low-lying plain soils in order to exploit the benefits provided by such management. More studies elucidating the mechanisms of greatest importance in improving soil structural conditions for silty soils, such as those examined in this study, are also required.

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