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# Impact of soil covering and tillage on soil physical-chemical quality

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# Summary

# Riassunto

La protezione del suolo, l'aumento della sostenibilità e del reddito degli agricoltori hanno contribuito ad un'ampia diffusione dell'agricoltura conservativa (CA). Tuttavia, in Europa l'adozione della CA è limitata, scoraggiata dai risultati mediocri ottenuti nel breve periodo. Il principale obiettivo di questa Tesi di Dottorato è di determinare, gli effetti combinati della riduzione delle lavorazioni e dell'introduzione di cover crops, nella fase di transizione da agricoltura convenzionale a conservativa. Si è ipotizzato che la combinazione di lavorazioni ridotte e il *tillage radish* come cover crop potesse minimizzare gli effetti indesiderati nella fase di transizione e, contestualmente, promuovere la fertilità e la funzionalità del suolo.

Per verificare questa ipotesi, è stata condotta una prova sperimentale triennale, nella bassa pianura veneta. Essa consisteva nella combinazione di tre intensità di lavorazione del suolo (lavorazioni convenzionali (CT), minima lavorazione (MT), e semina su sodo (NT)) con tre diverse gestioni della copertura del suolo durante l'inverno (suolo nudo (BS), cover crop di *tillage radish* (TR), cover crop di frumento (WW)).

In primo luogo, si è cercato di determinare gli effetti dei trattamenti sulle proprietà fisiche del suolo. Non tutti i parametri osservati hanno beneficiato dell'adozione della CA; tuttavia, si è riscontrato un miglioramento significativo di alcuni di essi nei regimi a lavorazioni ridotte. In particolare, la conduttività idraulica è risultata quattro volte maggiore in NT, se comparata agli altri trattamenti. Nel breve periodo, inoltre, sono stati osservati dei modesti benefici legati all'adozione di WW, mentre TR non ha prodotto effetti significativi. Questi risultati hanno dimostrato che per valutare correttamente la CA è necessario monitorare diversi parametri fisici del suolo, selezionandoli attentamente tenendo conto delle tempistiche di campionamento e della risoluzione degli strumenti.

In seguito, è stata misurata la sostenibilità complessiva del sistema, applicando l'analisi multivariata a una serie di indicatori di sostenibilità, ottenendo così un indice di sostenibilità relativa (Relative Sustainability Index - RSI). I sistemi a lavorazione ridotta e in particolare NT hanno riportato i valori più alti di RSI (+42% rispetto a CT e +13% rispetto a MT), anche se le rese si sono dimostrate più sensibili alle avversità, sia biotiche che meteorologiche. L'effetto delle cover crop è risultato limitato, ma si è osservata una tendenza positiva associata all'adozione di WW.

In ultimo, visti i modesti risultati legati all'introduzione di TR, si sono valutati gli effetti dell'epoca di semina (luglio, agosto, settembre, ottobre) sullo sviluppo di questa cover crop, i suoi effetti su parametri fisici del suolo e sulla sua sensibilità al gelo, attraverso un esperimento parcellare biennale. Per un'adeguata valutazione dei risultati ottenuti in TR, la si è confrontata con la senape bianca: una cover crop ben adattata all'agroecosistema del Nord Italia e sensibile al gelo. L'entità degli effetti osservati è stata limitata, tuttavia le cover crop seminate in settembre hanno raggiunto uno sviluppo adeguato, apportando alcuni dei servizi ecosistemici attesi.

Concludendo, i risultati ottenuti sono stati influenzati principalmente dalle lavorazioni. I sistemi a lavorazioni ridotte e in particolare NT hanno il potenziale per incrementare la sostenibilità ambientale ed economica delle aziende agricole, anche se sembrano più sensibili alle avversità. L'effetto delle cover crop si è dimostrato modesto, nondimeno se gestite correttamente, possono apportare benefici e servizi ecosistemici. Per una corretta valutazione di CA, è fondamentale un'attenta selezione degli indicatori da osservare.

Abstract

Farmers around the world have adopted conservation agriculture (CA) to protect soil, improve sustainability, and increase farm revenues. Despite these known benefits, inconsistent reports on the short-term results of CA discouraged its adoption in European agrosystems. To explore this topic in this Doctoral Project, studies were performed to determine the effects of different tillage intensity–soil covering combinations during the transition from conventional agriculture to CA. The starting hypothesis purported that the combination of reduced tillage systems and cover crop tillage radish (TR) can reduce the short-term drawbacks of CA and promote soil function and fertility.

To test this hypothesis, a three-year experiment in the low-lying Venetian plain (Northern Italy) was undertaken. It combined three tillage intensities (conventional tillage (CT), minimum tillage (MT), no tillage (NT)) with three winter soil coverages (bare soil (BS), TR, winter wheat cover crop (WW)).

The first phase evaluated the effects of the treatment combinations on soil physical properties. Results indicated that despite decline of some measures due to reduced tillage, the strategy enhanced soil physics. Specifically, hydraulic conductivity was four time higher under NT, if compared with other treatments. In the short term, cover crop WW increased physical soil parameters moderately, whereas TR showed negligible effects. The evidence demonstrated that CA effects require several soil physical parameters to be carefully selected and monitored while considering sampling time and resolution.

The second phase measured overall system sustainability by using a multivariate approach to calculate a Relative Sustainability Index (RSI) from a dataset of sustainability indicators. The manoeuvre showed that reduced tillage systems (NT, in particular) had the highest RSI values (+42% and 13% if compared to CT and MT respectively), but their yields were also especially prone to adverse biotic and meteorological conditions. Cover crop effects were limited, although WW tended to a positive RSI.

A third phase of research was designed to expand the limited TR results observed in the earlier phases. A two-year plot experiment was set up to evaluate several effects: seeding date (July, August, September, October) on TR development, TR effects on soil physical parameters, and TR frost sensitivity. White mustard was used as a comparison CC to assess the relative impact of TR; white mustard is well adapted to the agrosystem of Northern Italy and known to be killed in winter. The results from this phase showed that even when cover crop effects seemed limited, September-seeded TR and white mustard reached adequate development and provided some of the expected ecosystem services.

In conclusion, tillage intensity was the principal driver of results. The reduced tillage system, and NT in particular, seemed to have the potential to increase environmental and economic sustainability, even when it resulted as more susceptible to adverse conditions. Even though the size of the CC effect was limited, proper management can still provide soil benefits and ecosystem services. Careful selection of indicators seemed critical to assess CA effects correctly.

# Chapter 1

# General introduction

## Introduction

Conservation Agriculture (CA) is a management strategy aimed at increasing farm sustainability while simultaneously protecting the soil from threats and reducing production costs (Kassam *et al*. 2015). During the first years following conversion from conventional tillage to CA, referred to as the "transition time', negative results, such as yield reductions, have been well-documented and are now expected (Rusinamhodzi *et al*. 2011; Pittelkow *et al*. 2014; Piccoli *et al*. 2021). Topics in need of further description during this period include the actual duration of the period and the magnitude of its side effects as Piccoli *et al.* (2020) and Camarotto *et al.* (2020) observed in the Veneto Region.

Conservation Agriculture relies on three principles: 1) minimum soil disturbance, 2) permanent soil covering, and 3) crop rotation (FAO 2017). The first two will be elaborated below as they are most germane to this research. The most common strategies used to minimize soil disturbance are no-tillage (sod seeding) or minimum non-inversion tillage (Hobbs *et al.* 2008; Peigné *et al.* 2015). No-tillage is supported by a vast literature describing its positive effects on soil structure (Blanco-Canqui and Ruis 2018) and/or sustainability (Triplett and Dick 2008; Lal 2013). Similarly, minimum tillage can improve sustainability (Teodor *et al*. 2009), and under specific conditions, may be preferable to no-tillage (Borsato *et al.* 2018; Piazza *et al.* 2020). There are also instances in which occasional tillage may be the best choice, such as with contrasting-weed infestations (Liu *et al.* 2016; Chen *et al.* 2017) or when crop management involves compulsory soil tillage (Kirkegaard *et al.* 2014; Blanco-Canqui and Wortmann 2020).

The second CA principle—maintain a permanent soil covering—is easily observed by leaving crop residues on the soil surface (Baker *et al.* 1996). Alternatively, a cover crop (CC) grown between two main crops and then either leaving its biomass on the field or burying it before the subsequent cash crop provides the permanent soil covering and many other benefits that can increase CA sustainability (Schipanski *et al.* 2014). Selection of a CC species can be tailored for specific effects, such as weed suppression (Schappert *et al.* 2019), soil fertility and soil organic matter (SOM) improvement (Boselli *et al.* 2020), and/or soil physical parameter enhancement (Blanco-Canqui and Ruis 2020). By example, tillage radish (Raphanus raphanistrum sativus, L.) is an excellent CC when better soil physics are needed (Williams and Weil 2004).

Tillage radish, a Brassicaceous species, is characterized by a wide and deep taproot. When used as a CC, it is seeded after a summer crop, develops quickly (few weeks), and dies during a winter frost (Büchi *et al.* 2020). It can also benefit the subsequent crop by improving soil structure and facilitating conversion to reduced tillage (Toom *et al.* 2019; Wittwer and van der Heijden 2020). In fact, the use of tillage radish as a cover crop has been observed to produce a host of benefits: increased earthworm density (Euteneuer *et al.* 2019), enriched soil nutrient dynamics (Zhao *et al.* 2020; Norberg and Aronsson 2020), improved system sustainability (Crotty and Stoate 2019; Ciaccia *et al.* 2019), and supressed weeds (Schappert *et al.* 2018, 2019; Sturm *et al.* 2018; Ranaldo *et al.* 2019). In Northern Italy, tillage radish CC cultivation is limited and information about its use is scarcely distributed. However, its use in CA managements indicates its potential to counter some transition time common effects, such as reduced soil porosity and increased soil strength and bulk density, which have been observed locally by Dal Ferro *et al.* (2014) and globally by Lipiec *et al.* (2006); Mentges *et al.* (2016); Martínez *et al.* (2016).

Worldwide, the proven benefits of CA to protect soil and to provide several ecosystem services (Palm *et al.* 2014) have been borne out by its use on 12.5% of all cultivated land. Unfortunately, the growth of CA across the globe has been applied to a lesser degree in Europe (5%), and nearly unutilized in Italy (Kassam *et al.* 2019).

In the micro-structured soils of the low-lying Venetian plain, reduced Soil Organic Matter (SOM) accompanied by soil compaction was observed (Piccoli *et al.* 2020) has resulted in the advice that CA is one strategy to oppose these two soil threats (Morari *et al.* 2006). Based on the ability of CA to foster soil C protection and sequestration, to limit global warming, and to promote environmental sustainability (Lal 2004) while improving soil structure (Thomas *et al.* 1996; Hobbs 2007) has also led Rural Development Programs (Regione Veneto 2014) to endorse the practice. The lukewarm diffusion of CA in Europe may arise from the limited positive effects observed during the transition, which may last for more than five years (Camarotto *et al.* 2020). During this extended period, yield reduction and other effects on the physical parameters of soil have been reported in a variety of European agroecosystems (Munkholm *et al.* 2003; Buczko *et al.* 2006) and locally (Piccoli *et al.* 2017b, a). Limited CA effects have also been observed on C stocks (Piccoli *et al.* 2016; Longo *et al.* 2020), which seems to contrast with the Smith *et al.* (1998) notion that CA is a potential strategy to mitigate agriculture C emissions and attain farm C neutrality.

To appreciate these contrasting results required site-specific trials that combines different tillage intensities with different soil coverings. System sustainability of the systems was assessed by monitoring and measuring short-term soil property changes. Evaluation of the short-term effects after conversion to CA in the low-lying Venetian plain agroecosystem may reveal the best combination of factors to maximize benefits and mitigate drawbacks.

## Objective and outline

This PhD project had three main objectives: 1) to monitor physical and biochemical indicators during transition from conventional agriculture to Conservation Agriculture (CA); 2) to identify the effects of various tillage intensity—cover crop (CC) combinations; 3) to evaluate the development and effects of a specific CC (tillage radish: Raphanus raphanistrum sativus, L.) in the Veneto Region agroecosystem.

The study series started with the hypothesis that by combining reduced tillage systems with tillage radish cover crop (TR), short-term drawbacks of CA could be reduced, improved soil function and fertility could be promoted, and the claims of negative effects from CA could be clarified. To test this hypothesis required design and set-up of a large-scale field experiment to compare various treatment combinations derived from three tillage intensities (conventional inversion tillage, minimum non-inversion tillage, and no tillage) and three different soil covering managements (crop residues soil cover, tillage radish cover crop, and winter wheat cover crop). Winter wheat was selected as its fibrous root apparatus is relevant for comparison with tillage radish taproot.

Chapter 1 discusses the soil physics research undertaken to address reports of declines in some physical parameters during conversion to CA. It was hypothesized that reduced tillage systems would show a general worsening in these parameters, except when combined with tillage radish. Several indicators were selected to assess the coupled evolution of soil compaction and soil function at scale. Specifically, estimates were prepared for three pairs: soil strength with penetration resistance, soil porosity with bulk density, and soil-water dynamics with saturated hydraulic conductivity.

Chapter 2 discusses the analysis of different sustainability indicators used to evaluate the effects of the treatment combinations from physical, chemical, and biological perspectives. This work led to the development of a sustainability index capable of determining not only the best treatment combination, but also pivotal parameters that impact system evaluation most.

The need for a deeper understanding of tillage radish development and its effects was revealed in the preliminary results collected during the first year of experimentation. A targeted two-year plot experiment was conducted, and those results are presented in Chapter 3. The hypothesis for this put forth that seeding date may significantly affect tillage radish development, frost sensitivity, and soil effects. To test this hypothesis, tillage radish was seeded on four different dates. For comparison purposes, white mustard (*Sinapis alba*, L.), a Brassicaceous CC with similar agronomic characteristics and fully adapted to the growing conditions at the site, was identically sown.

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# Chapter 2

# Transition to conservation agriculture: how tillage intensity and covering affect soil physical parameters

# 1 Introduction

Minimal soil disturbance, permanent soil covering, and crop rotation represent the main pillars of Conservation Agriculture (CA) (FAO 2017). Adoption of CA not only leads to reduced labour and farm costs, but also provides several ecosystem services that increase agroecosystem sustainability. Its hallmarks of reduced soil tillage, applied cover crops (CC), and rotated crops generally improve the physical parameters of soil and foster nutrient cycling and soil biological activity. In general, CA has been shown to enhance most soil physical properties, but some contrasting results have been reported (Blanco-Canqui and Ruis 2018). Negative outcomes have often been obtained in no tillage (NT) systems that failed to specify whether or not the soil was permanently covered between two main crops. Typically, CCs are used to maintain soil coverage. It consists of cultivating plants between two main crops, leaving the entire biomass on the field after the growing season, and eventually burying it before the subsequent crop is planted (Schipanski *et al.* 2014). The use of CC is a pivotal strategy for enhancing soil physical properties in reduced tillage systems (Blanco-Canqui *et al.* 2011).

Despite a growing interest in CA from many agroecosystems and especially in the Americas, European adoption of the practice has faltered (Kassam *et al.* 2019). One reason behind limited CA adoption in Europe is uncertainty about its effects during the transitional period after conversion from conventional to conservation agriculture (Rusinamhodzi *et al.* 2011; Pittelkow *et al.* 2014). Site-specific trials offer not only a chance to expand what is known about the impact of CA on soil physical parameters, but also an opportunity to determine an optimal tillage—CC combination capable of mitigating local soil threats while simultaneously reducing conversion-time side effects. Indeed, under specific conditions, occasional tillage is recommended (Liu *et al.* 2016), whereas in other situations, implementation of minimal tillage (MT) may provide benefits equal to those of NT (Teodor *et al.* 2009; Chen *et al.* 2017). Moreover, efficient use of CC requires careful selection of species, seeding date, and management strategy (Daryanto *et al.* 2018). Differing species may positively impact nutrient cycling, soil properties, and/or weed suppression, although such factors must be cost-effective, since they do not contribute directly to profitability (Schappert *et al.* 2018; Ranaldo *et al.* 2019).

In the low-lying Venetian Plain of Northern Italy, soils contain low organic carbon, high carbonate, and are micro-structured. The principal threats to such soils are organic matter depletion and compaction (Piccoli *et al.* 2020). Traditionally, farmers have countered compaction with annual deep ploughings that, in the long-term, may contribute to plough pan formation and foster organic matter mineralization. Among the benefits of CA is its potential to improve soil structure along the full soil profile, while protecting soil organic matter (Thomas *et al.* 1996; Hobbs 2007). Nonetheless, contrasting results have been reported, especially in the early years after CA adoption. In general, negative reports of the short-term effects of CA on physical soil parameters seem limited to bulk density (Guan *et al.* 2014), soil strength (Munkholm *et al.* 2003; Palm *et al.* 2014), and soil saturated hydraulic conductivity (Buczko *et al.* 2006). The use of CC to minimise the side effects of NT or MT represents a valuable short-term solution to facilitate conversion from conventional agriculture to CA. If cash crops are grown during the spring and summer, then autumn-drilled CC must develop rapidly to cover the soil before winter, and devitalisation must occur in the spring before cash crop seeding.

Suitable CC species for northern Italy agroecosystems are *Poaceae* species (e.g., wheat, barley, oat, ray, and triticale), which already is well adapted and easily managed by farmers. *Poaceae* can control weeds and reduce nutrient losses. Moreover, its fibrous root apparatus can positively impact soil physical properties, especially in the shallow soil layer

(García-González *et al.* 2018). Alternatively, to mitigate soil compaction and improve the physical quality of the soil, tillage radish (*Raphanus raphanistrum sativus* L.) "TR" has been broadly applied as a CC (Crotty and Stoate 2019; Ciaccia *et al.* 2019). TR is a brassicaceous plant, specifically selected to improve the macro-porosity and pore connection of soil. Its 5 cm (D) × 30 cm (L) taproot counters soil compaction while enhancing water infiltration. While it is killed in the winter, it is easily managed in the spring (in a NT system also) (Büchi *et al.* 2020). As has been demonstrated by the limited use of CC throughout northern Italy, there is a general lack of knowledge on TR adaptability in such agroecosystems, and its effectiveness at improving soil properties.

The evolution of soil physical traits is frequently done by measuring soil Bulk Density (BD), soil Penetration Resistance (PR) and soil infiltration (Blanco-Canqui and Ruis 2020). These indicators of soil strength, soil porosity and water and gas permeability are evaluated from measures using different scales. Specifically, PR is evaluated most often using penetrometers having probes of a few centimetres in diameter, BD is determined from undisturbed soil core samples having a slightly larger diameter, and soil infiltration measures typically rely on infiltrometers of a far larger size (Al-Shammary *et al.*, 2018; Dexter *et al.*, 2007; Morbidelli *et al.*, 2017). These different measurement scales can greatly affect results, particularly in no-till soils, where not only root penetration, but water and gas penetration, can be principally affected by the presence of bio-pores that create preferential pathways for root development even in what seem like highly-compacted soils. The goals of this study are to evaluate soil physical traits using these different spatial resolution measures during the transition from conventional tillage to CA. For this purpose, BD, PR, and soil hydraulic parameters were monitored from 2018 to 2020 in field surveys conducted on trials created by combining three different tillage system with three winter soil coverings.

## 2 Materials and methods

The experiment took place at the Lucio Toniolo Experimental Farm, located in Legnaro, PD (NE Italy, 45° 21 N; 11° 58 E; 6 m a.s.l.), where the climate is sub-humid, with temperatures between -1.5 °C on average in January and 27.2 °C on average in July. Rainfalls reach 850 mm annually, with a reference evapotranspiration of 945 mm that exceeds rainfalls during April to September. Highest rainfalls occur in June (100 mm) and in October (90 mm), while winter is the driest season with average rainfalls of 55 mm. The shallow water table ranges from 0.5 to 2 m in depth, with the lowest values recorded in summer.

The trial, begun in spring 2018, was designed as a split plot, with two replicates. A 2 ha area was divided into 18 plots of 1,111 $m^2$ each. Soil at the site is Fluvi-Calcaric Cambisol (FAO-UNESCO 2008) with a silty loam texture.

At the start of the experiment, the average soil texture of each plot was determined by laser diffraction (Malvern Mastersizer 2000; Malvern Instruments, Malvern, UK) as described in Bittelli *et al.*, 2018. Three different tillage treatments were randomized in plots: the conventional tillage (CT) plot was ploughed to 30 cm and harrowed (15 cm), the minimum tillage (MT) plot was tilled to a depth of 15 cm and then harrowed, and the no tillage (NT) plot was sod-seeded. The first application of different tillage intensities was performed at the beginning of the experiment, in spring 2018, before the main crop seeding. Then, three winter soil coverings were randomized within each of these plots after the main crop: TR (*Raphanus raphanistrum sativus* L.), winter wheat (WW – *Triticum aestivum* L.), and bare soil (BS), where no soil cover was present other than the residues from the crop of the previous year. Cover crops were drilled on the main crop residues in autumn 2018 and 2019 with a sod seeding driller. The seed density was equal to 9 kg $ha^{-1}$ for

TR and 150 kg ha$^{-1}$ for WW. Both CC were devitalized in spring, when they reached an average biomass of 1.1 Mg ha$^{-1}$; then the main crop was always maize (*Zea mays* L.), during its growing season drip irrigation was performed in 2019 and 2020. Extreme meteorological conditions together with the consistent bird damage were observed in spring 2019. This led to the maize crop failure, after which it was reseeded in May.

## 2.1 Field surveys

Four parameters were selected to monitor soil physical qualities: bulk density (BD), penetration resistance (PR), and saturated hydraulic conductivity (Ks) together with sorptivity (S). The survey timetable is shown in Figure 1. The sampling dates were selected to monitor the soil evolution. Noteworthy is the fact that PR required adequate soil condition to be performed (i.e., sufficiently wet soils), while BD and hydraulics are destructive measures which require field accessibility.

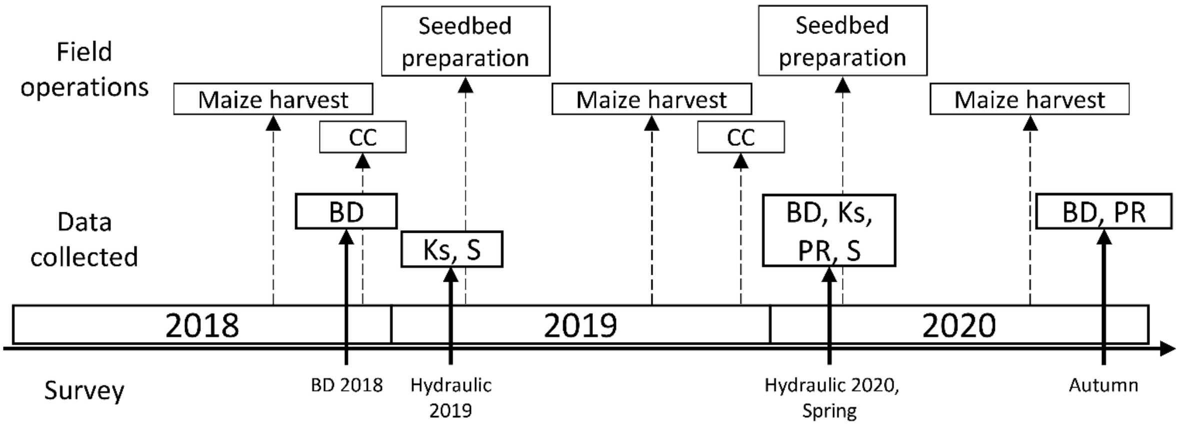


**Figure 1. Survey timetable. BD: bulk density, CC: cover crop seeding, Ks: saturated hydraulic conductivity, PR: penetration resistance, S: sorptivity**

### 2.1.1 Bulk density

The surveys were conducted on three sampling dates. Measurements were first performed at the start of the experiment after the first-year harvest (BD 2018, time 0). The second collection occurred in 2020 before tillage operations and after CC devitalization, in May. The final sampling was performed in the same year, after the maize harvest but prior to soil preparation and subsequent crop seeding in November. Hereafter, the first, second, and third BD surveys will be referred to as “2018”, “Spring”, and “Autumn”, respectively. Each soil core was considered in 10 cm layers, which yielded six different depth-linked BD values from each sample. All samples were oven dried (24 hr at 105°C) to calculate BD (core method) (Grossman and Reinsch 2002) on undisturbed 7 cm diameter soil cores that were collected with a hydraulic probe from the 0-60 cm layer.

### 2.1.2 Penetration resistance

Penetration Resistance (PR) was measured with a penetrologger (Eijkelkamp, Netherland) throughout the 0-60 cm layer with a 30° 2 cm$^2$ cone. In each plot, four sampling zones were randomly selected. In each sampling zone, four penetration measures were performed within an area of 0.25 m$^2$. Disturbed soil samples were also collected to determine gravimetric water content and soil texture in each 20 cm soil layer (0-20, 20-40, 40-60, and 60-80 cm). The penetrologger measured from 0 to 5 MPa. Two PR samplings were performed in the same fashion in the Spring and Autumn surveys as described above, and coincident with the second and third BD measures (Figure 1). PR values were averaged for each 10 cm of the soil profile and compared with the 2.5 MPa threshold considered a critical value above which root growth may be compromised according to Groenevelt *et al.* (2001).

### 2.1.3 Saturated hydraulic conductivity and sorptivity

Saturated hydraulic conductivity (Ks) and sorptivity (S) parameters were measured by a double-ring infiltrometer on an area of 1,300 $cm^2$, as described in Morbidelli *et al.* (2017). Ks measures the water column that can infiltrate in a soil under saturated conditions, in the time unit, while S is the early time infiltration, when the soil is under unsaturated condition, and it is dominated by capillary forces (Cook and Broeren 1994). Philip's equations (Philip 1969) were fitted to the field data to calculate Ks and S. Two surveys (spring 2019 and spring 2020) were conducted to measure these parameters after CC termination and before soil preparation. These surveys were conducted in March 2019 and May 2020.

### 2.1.4 Meteorological data and yield

Meteorological data were monitored during the three-year experiment. This information was obtained from an ARPAV (Regional Agency for Environmental Protection and Prevention of Veneto) weather station located 100 m from the trials. These meteorological data (namely temperature and rainfall) were strictly related with the cash crop yield performances, which were measured through grain biomass collection at the end of the maize cycle.

### 2.2 Statistical analyses

A mixed-effects model was applied to test the main effects of tillage, soil covering, and their interactions on all i-th variables for each monitoring period. The sand content was considered a covariates, together with BD in Ks, S and PR models. All effects named above were treated as fixed effects; the block effect was treated as random and the replicate measurements inside the same plot were considered as nested. All possible first and second order interactions between factors were tested, and the model with the smallest AIC (Akaike's Information Criterion) was selected (Schabenberger and Pierce 2001). Post hoc pairwise comparisons of least squares means were performed using the Tukey method to adjust for multiple comparisons. A similar procedure was applied to test the effects of year and treatment combinations on yield.

For penetration resistance, the percentage of measures above 2.5 MPa with the whole soil profile considered was tested with Kruskal-Wallis ANOVA, as these data were not-normally distributed. The BD-PR correlation significance was F-tested. All statistical analyses were performed with SAS (SAS Institute Inc. Cary, NC, USA) version 5.1.

## 3 Results

### 3.1 Bulk density

The first BD survey was conducted at the beginning of the experiment (time 0). At that time, no differences were observed among the plots. In particular, BD ranged between 1.14 and 1.60 g $cm^{-3}$ (average value of 1.40 g $cm^{-3}$) in the tilled layer (0-30 cm). In the deepest layer (30-60 cm), the mean value was higher at 1.49 g $cm^{-3}$ within a range of 1.30 g $cm^{-3}$ and 1.69 g $cm^{-3}$. No statistical differences were observed (Fig. 2, Table 1).

On the contrary, significant differences were observed in the 2020 Spring survey. In the 0-30 cm soil layers, the CT-BS treatment combination displayed the lowest average BD value (1.37 g $cm^{-3}$, or 5.1% lower) among all other treatments.

In NT, cover crops TR and WW both reduce BD values in the 10-40 cm layer (1.54 g cm$^{-3}$ on average) when compared to BS (1.58 g cm$^{-3}$). Generally, a tillage effect was prevalent in the 10-30 cm soil layer (Fig.2). Consistently, CT average BD was 1.37 g cm$^{-3}$, as opposed to the 6.5% higher values found in the same layer in MT and NT. In the deepest layer, BD values were even higher, ranging from 1.54 g cm$^{-3}$ to 1.91 g cm$^{-3}$. Here, the reduced tillage systems proved to reduce BD moderately, whereas CC produced limited results.

The Autumn BD survey exhibited a greater tillage effect along the soil profile relative to the time-zero survey. Bulk density results in the 0-10 cm layer of NT differed markedly from other treatments, except MT-WW. Indeed, they averaged 6.6% above (1.46 g cm$^{-3}$) the others. In these cases, the presence of a cover raised BD values throughout the soil profile by 2.9% (1.41 g cm$^{-3}$). In the subsequent soil layer (10-20 cm), CT showed the lowest average BD values (1.43 g cm$^{-3}$), whereas at depths below 20 cm (20-60 cm), CT treatment resulted in 2.2% higher average BD values (1.57 g cm$^{-3}$) when compared to the reduced tillage systems (MT and NT). Again, the CC effect seemed limited as TR and WW showed 2.8% higher BD (1.48 g cm$^{-3}$) values in the 0-30 cm layer.

**Table 1. Comparison of p values among the linear mixed-effect models analysis of bulk density (BD), penetration resistance (PR), saturated hydraulic conductivity (Ks), and sorptivity (S). Effects were considered significant if $p \le 0.05$.**

| | BD | | | PR | | Ks | | S | |
|---|---|---|---|---|---|---|---|---|---|
| | 2018 | Spring | Autumn | Spring | Autumn | 2019 | 2020 | 2019 | 2020 |
| Intercept | 0.0329 | 0.008 | 0.007 | 0.095 | <0.001 | 0.207 | 0.155 | 0.123 | 0.118 |
| Tillage | 0.8849 | <0.001 | 0.003 | <0.001 | 0.034 | <0.001 | <0.001 | <0.001 | <0.001 |
| CC | 0.0952 | <0.001 | <0.001 | 0.738 | 0.002 | <0.001 | 0.026 | <0.001 | <0.001 |
| Tillage*CC | 0.6640 | <0.001 | <0.001 | 0.006 | 0.014 | <0.001 | <0.001 | <0.001 | <0.001 |
| BD | # | # | # | 0.280 | 0.369 | -- | -- | -- | -- |
| sand | 0.4293 | <0.001 | 0.573 | <0.001 | 0.041 | 0.2002 | 0.0188 | <0.001 | <0.001 |
| Depth | 0.0000 | <0.001 | <0.001 | <0.001 | <0.001 | # | # | # | # |
| Tillage*Depth | 0.5307 | <0.001 | 0.001 | 0.003 | <0.001 | # | # | # | # |
| CC*Depth | 0.9638 | <0.001 | <0.001 | -- | -- | # | # | # | # |
| Tillage*CC*Depth | 0.9932 | <0.001 | <0.001 | -- | -- | # | # | # | # |
| GWC | # | # | # | 0.404 | 0. 002 | # | # | # | # |

-- effect not included in the model according to the Akaike Information Criterion; # not applicable.

## 3.3 Penetration resistance

Results indicated that soil structure, soil texture, and soil water content each affected PR in both 2020 surveys (Table 1). Conditions were, on average, drier during the Autumn survey (16.3% gravimetric water content) than during the Spring survey (22.2% kg kg$^{-1}$), for which average PR values were 2.52 MPa and 1.58 MPa, respectively. During both surveys, significant tillage × depth and tillage × CC interactions were detected (Table 1). A comparison among the three tillage systems showed that CT exhibited lower PR values than MT and NT in the 10 to 30 cm depth in both surveys (Fig. 3). Indeed, CT reported average PR values of 1.04 MPa (Spring) and 1.91 MPa (Autumn), while the reduced tillage treatments increased their PR values +35.6% (1.41 MPa) in Spring survey and +31.4% (2.51 MPa) in Autumn survey.

When the entire soil profile was considered, CT (regardless of the winter soil covering), as well as MT-TR and NT-BS were associated with the lowest PR values, in Spring survey (1.50 MPa, on average, Fig. 4). The highest PR value occurred in MT-BS (1.74 MPa). Alternatively, in Autumn, the highest PR was measured in MT-TR (2.81 MPa), while MT-BS, CT-WW, CT-BS, and MT-WW (on average 2.42 MPa) were all among the lowest. CT-TR and the NT treatments resulted in intermediate PR values that ranged between 2.51 MPa (NT-WW) and 2.55 (NT-BS).

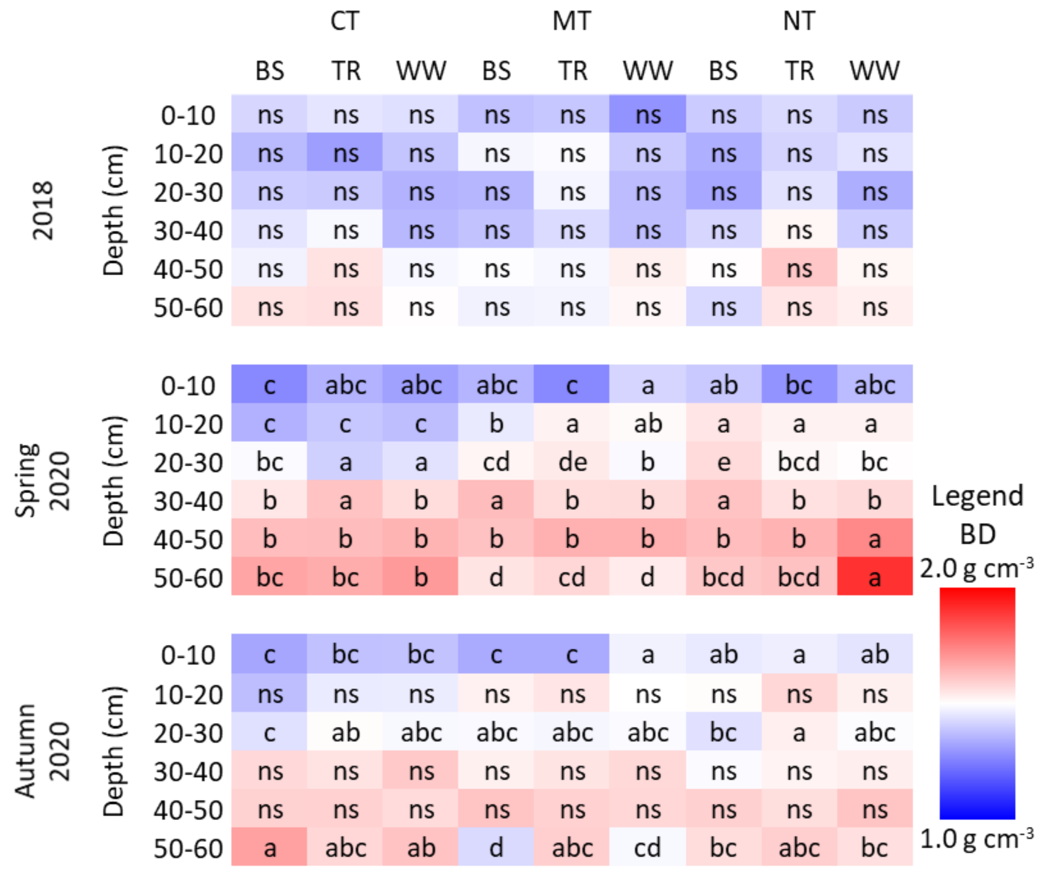


**Figure 2. Bulk density (BD) distribution along the 0-60 cm soil profile. For each soil layer, the letters indicate significant effects of tillage x CC according to the Tukey test ($p<0.05$). CT: conventional tillage; MT: minimum tillage; NT: no-tillage; BS: bare soil; TR: tillage radish; WW: winter wheat.**

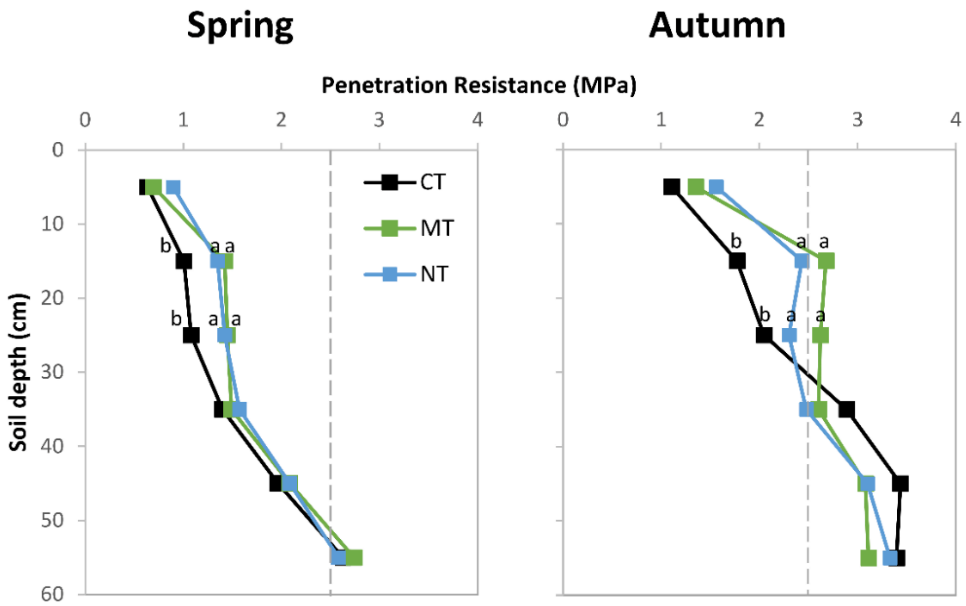


**Figure 3. Penetration resistance (PR) along the 0-60 cm soil profile (values averaged every 10 cm). Different letters represent significant differences according to the post-hoc Tukey test (p<0.05). The vertical dashed line indicates the 2.5 MPa threshold according to Groenevelt *et al.* (2001). CT: conventional tillage; MT: minimum tillage; NT: no-tillage.**

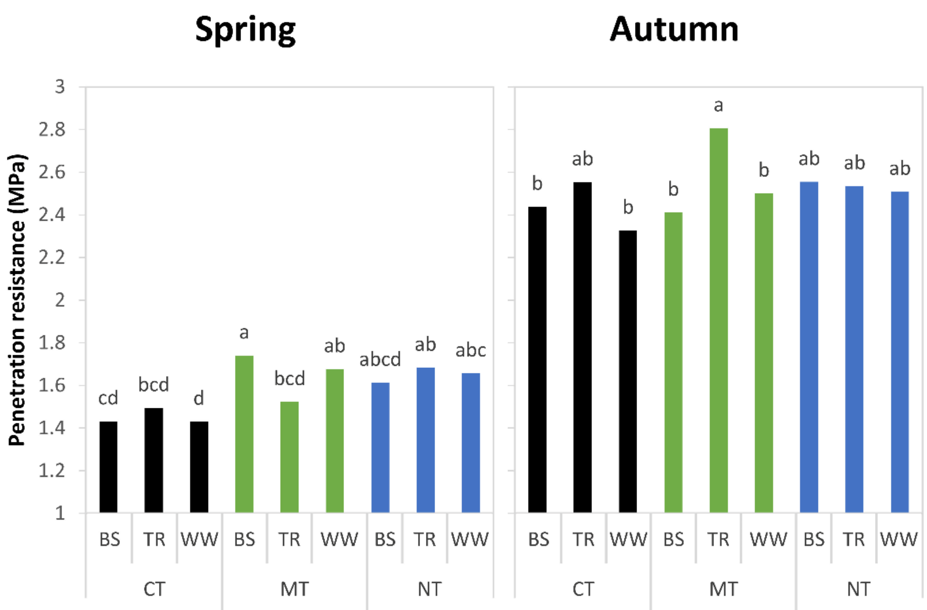


**Figure 4. Penetration resistance along the 0-60 cm soil profile. Different letters represent significant differences according to the post-hoc Tukey test with p<0.05. CT: conventional tillage; MT: minimum tillage; NT: no-tillage; BS: bare soil; TR: tillage radish; WW: winter wheat.**

The PR values were then compared with the 2.5 MPa threshold (Fig. 5). During the first survey (Spring) only 13% of measures were above this threshold, mostly beneath the tilled layer. During the Autumn survey, the proportion of measure above the threshold rose to 46%, with a high percentage reported throughout the full soil profile. The Kruskal-Wallis one-way ANOVA indicated there was a significant (p<0.05) effect related to the combination of tillage and CC. Close examination showed that the MT-TR treatment combination resulted with the highest proportion of over-threshold PR values (60%). It was followed by NT-BS (53%) and all the other treatment combinations ranged between 41% and 45%.

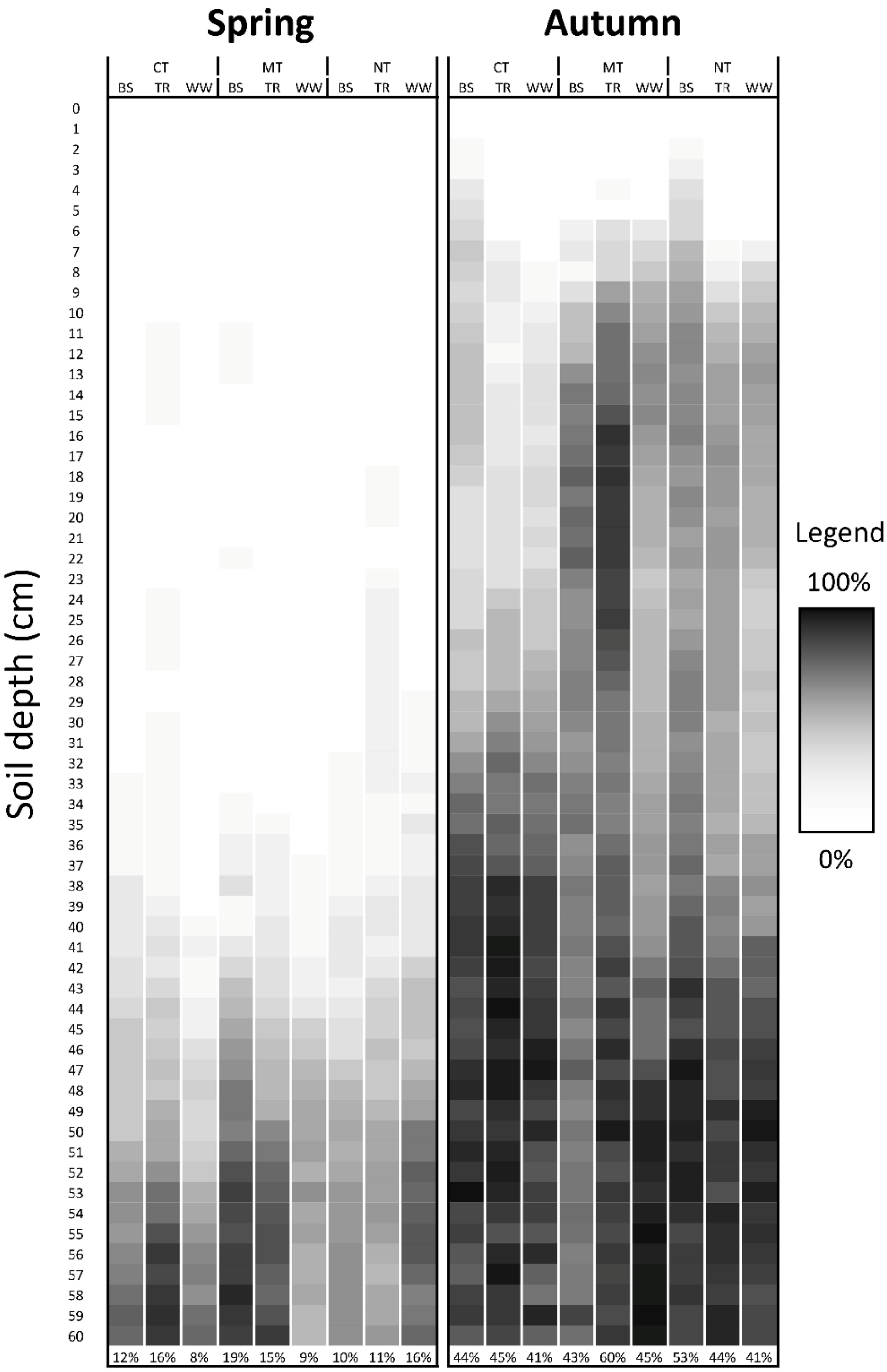


**Figure 5. Percentage of penetration resistance measures above the 2.5 MPa threshold. CT: conventional tillage; MT: minimum tillage; NT: no-tillage; BS: bare soil; TR: tillage radish; WW: winter wheat.**

## 3.4 Soil hydraulic properties

A significant tillage × CC interaction effect was observed on Ks during both the 2019 and 2020 surveys (Fig. 6). The combination of NT-WW produced the highest 2019 Ks value, which represented a two-fold increase compared to all other treatments ($2.50 \times 10^{-5}$ m $s^{-1}$ *vs* $1.04 \times 10^{-4}$ m $s^{-1}$, respectively). During the 2020 survey, all treatments exhibited increased Ks values that were 1.6 times higher, on average, than those of 2019. In particular, the combination of either BS or WW with NT, had the highest Ks ($2.12 \times 10^{-4}$ m $s^{-1}$), which was more than four time the values of all other treatments ($5.14 \times 10^{-5}$ m $s^{-1}$, on average). It is worth noting that TR displayed no effect in any combination in either year.

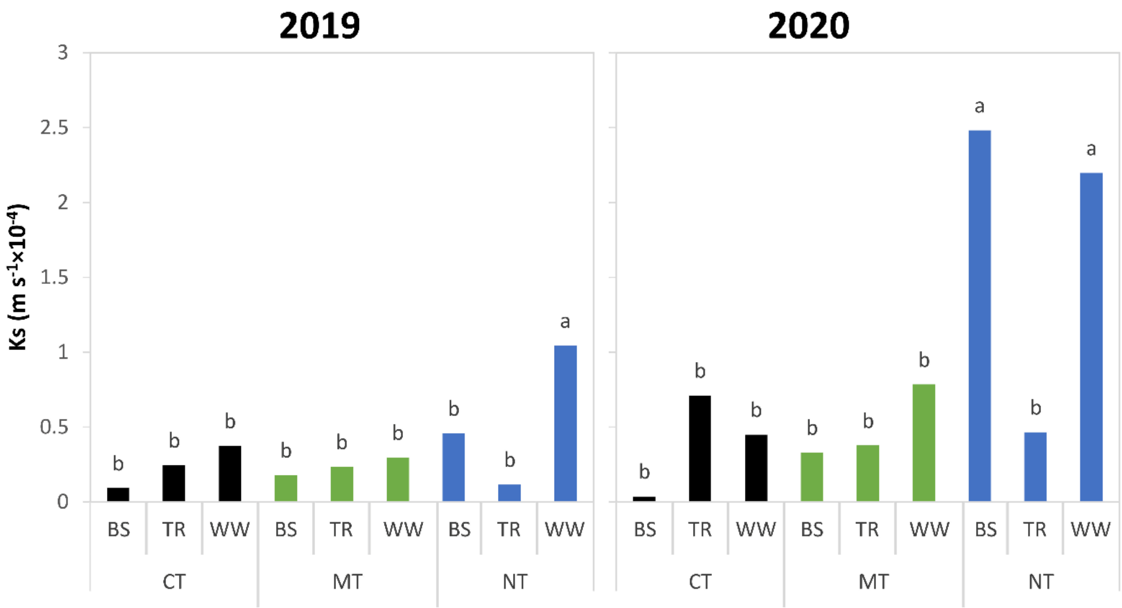


**Figure 6. Saturated hydraulic conductivity (Ks) as measured in the two surveys (2019 and 2020). Different letters represent significant differences according to the post-hoc Tukey test ($p<0.05$). CT: conventional tillage; MT: minimum tillage; NT: no-tillage; BS: bare soil; TR: tillage radish; WW: winter wheat.**

Sorptivity (S) was affected both by the interaction of tillage × CC and soil texture (Table 1, Fig. 7); sand content negatively correlated with S. Identical tendencies were observed in both years. Among the various treatment combinations, NT-BS reported the highest results $1.27 \times 10^{-4}$ m $s^{-1}$ (2019) and $3.19 \times 10^{-5}$ m $s^{-1}$ (2020). Very low values of S were observed in CT-BS ($8.5 \times 10^{-7}$ m $s^{-1}$, on average) during the 2020 survey.

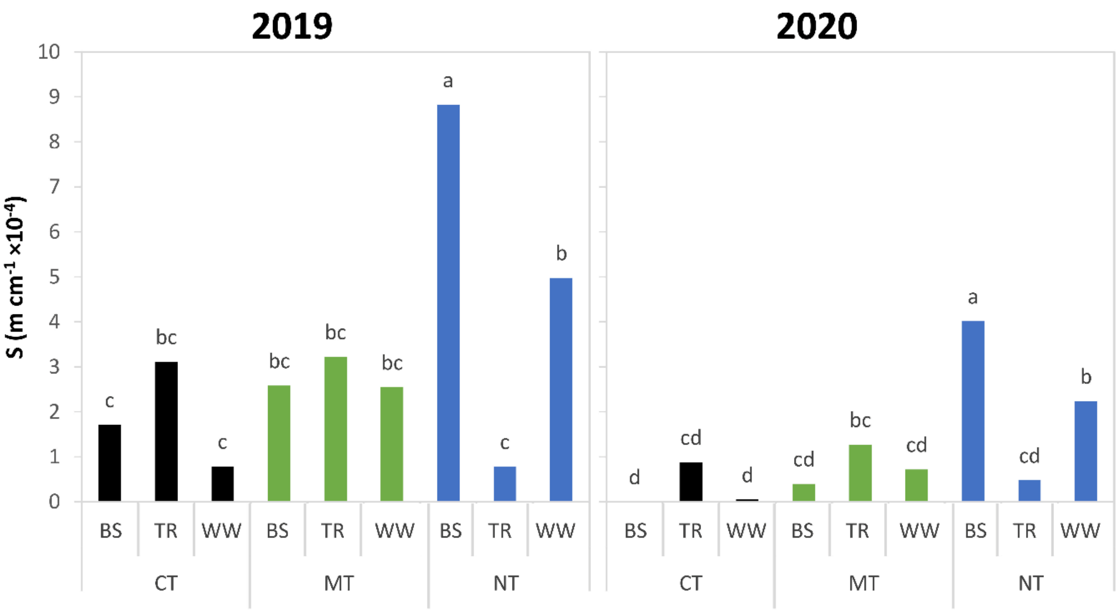


**Figure 7. Sorptivity (S) in the two surveys (2019 and 2020). Different letters represent significant differences according to the post-hoc Tukey test ($p<0.05$). CT: conventional tillage; MT: minimum tillage; NT: no-tillage; BS: bare soil; TR: tillage radish; WW: winter wheat.**

## 3.5 Correlation between bulk density and penetration resistance

A significant ($p<0.01$) positive linear relationship was found between BD (range of 0.5-2.5 MPa) and PR (range of 1.33-1.80 g cm$^{-3}$) with 0.36 $R^2$. At a PR> 2.5 MPa, the correlation with BD was lost; and no other regression could be found between the two parameters. At points above the critical limits of PR (2.5 MPa) and BD (1.55 g cm$^{-3}$), 46% of the observations were detected in CT, 31% in MT, and only 23% in NT, as the red box highlights in Fig. 8. Under these limiting conditions, WW reported the fewest number of measures above this threshold. Following WW was BS; TR had 35% of observations in the range.

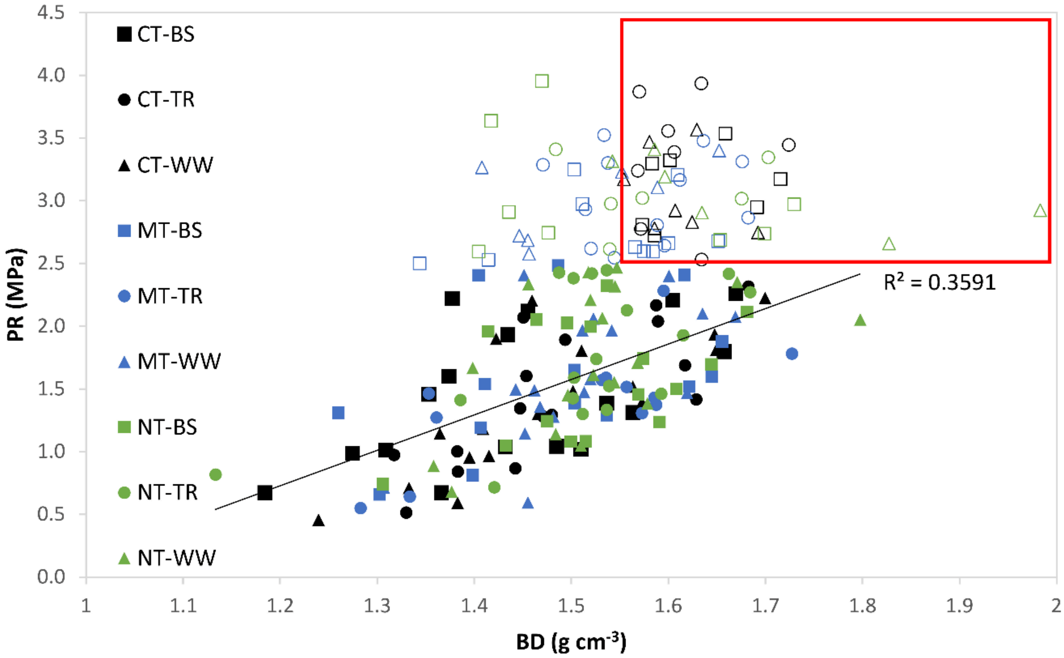


**Figure 8. Linear regression between bulk density (BD) and penetration resistance (PR). The line represents the significant (p<0.01) linear regression for PR<2.5 MPa and BD <1.8 g cm$^{-3}$. Closed and open indicators are used for PRs below or above 2.5 MPa, respectively. The red box highlights observations above both 1.55 g cm$^{-3}$ BD and 2.5 MPa PR.**

## 3.6 Meteorological trend and grain yield

Monthly rainfall and monthly average temperature are shown in Fig. 9. It is worth to note the extreme rain event registered in May 2019, that reported a total monthly rainfall of 200 mm. These adverse conditions, negatively impacted on maize growth, with a stronger impact on NT yield (Fig.10). In fact, no significant differences were observed in maize yield among treatment combinations, both in 2018 and in 2020, which reported an average value of 10.00 Mg ha$^{-1}$. Instead, in 2019 all treatment reported significant lower value (on average 5.10 Mg ha$^{-1}$). Among the treatment NT yield resulted significantly lower (1.49 Mg ha$^{-1}$) than CT and MT (6.91 Mg ha$^{-1}$, on average).

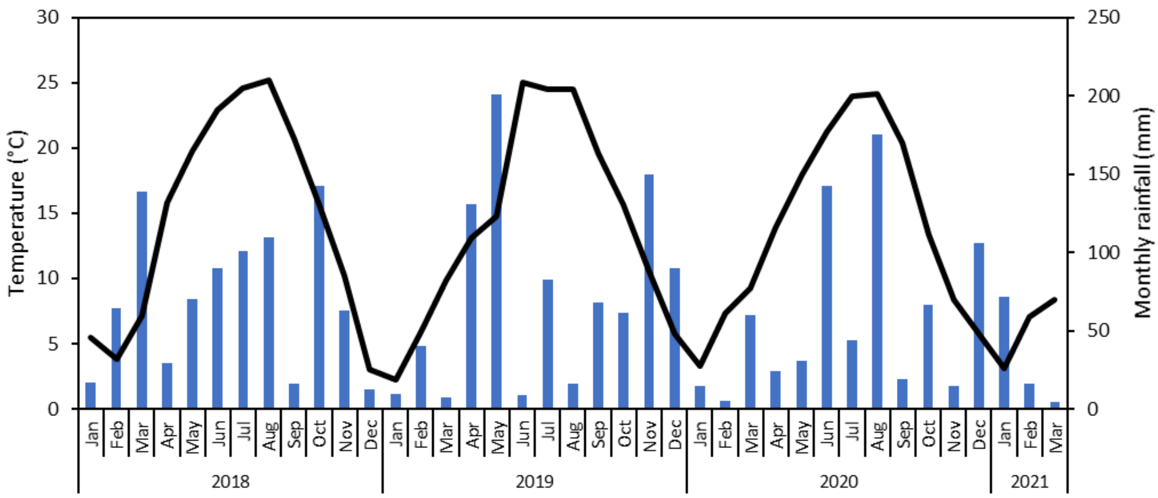


**Figure 9. Average monthly temperature (black line) and Monthly rainfall (blue bars).**

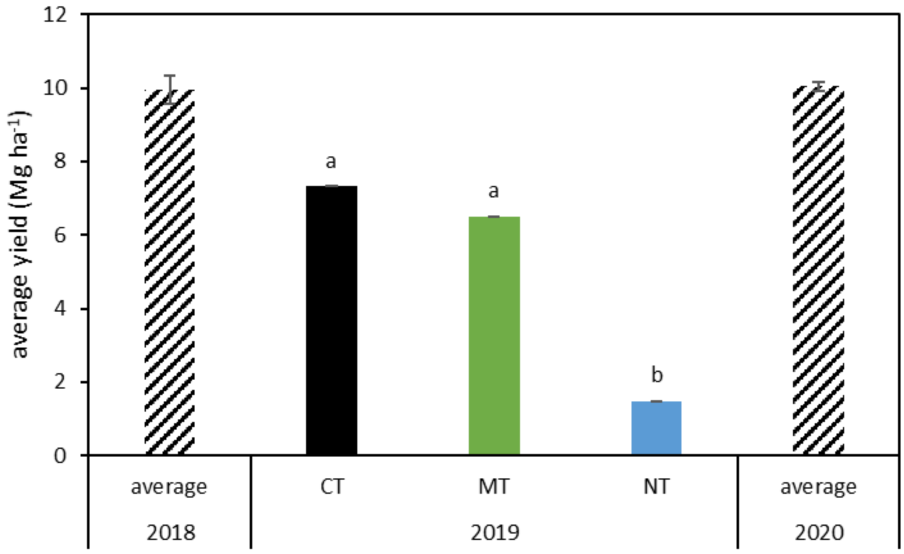


**Figure 10. Average yield in the three experimental years. Different letters represent significant differences according to the post-hoc Tukey test ($p<0.05$). CT: conventional tillage; MT: minimum tillage; NT: no-tillage.**

## 4. Discussion

In the system monitored, the transition from conventional to conservation agriculture did not affect the potential growth of the cash crop: no significant differences among the three tillage treatments were reported in yield, the first and third year from conversion. However, MT and NT systems proved to be more susceptible to adverse conditions. Particularly with NT, the agronomic techniques such as sowing are critical and can lead to a bad emergence of the crop in specific meteorological conditions. In 2019, sowing was delayed by a heavy spring rainfall and, moreover, the crop was subjected to a consistent bird damage. In consequence a consistent yield reduction was observed in NT, in comparison to both MT and CT. This result stress the importance of a proper management of the fallow period, in particular if cover crops are present, to avoid the risk of delaying the subsequent cash crop sowing or to allow an uncontrolled germination of weeds in NT.

The results presented above confirmed that employing a combination of tillage and CC has limited effects in the short term, as Perego *et al.* (2019) and Piccoli *et al.* (2017a) previously reported in similar agroecosystems. Nonetheless, initial, short-term effects on soil physical parameter can be detected in some situations by measuring BD, PR, and soil hydraulic properties. Driven primarily by tillage intensity, lower BD values were found in the tilled layer of both CT and MT. Furthermore, the results highlighted that the magnitudes of BD values at the deeper levels of soil tillage (30 cm ploughing) were similar to those at shallower tillage depths (≤ 15 cm). This finding is consistent with work by Guan *et al.* (2014). According to the USDA Natural Resources Conservation Service (1996), a BD value of 1.55 g $cm^{-3}$ in silty loam soils represents a threshold above which plant growth may be hindered. In this study, this threshold was exceeded, especially at depths below the tilled layer in the first survey (2018), which may be linked to the presence of a plough pan that arose due to repeated soil tillage to the same depth. In a similar agroecosystem, the presence of a plough pan was detected when geophysical and direct assessment methods were combined (Piccoli *et al.* 2020). Specifically, the authors found the plough pan responsible for shallower and greater lateral development of the root apparatus in winter cereals, although it seemed not to affect spring crops (maize, soybean) (Piccoli *et al.* 2021). During the last survey of the study, both MT and NT exhibited lower BD values beneath the tilled layer. This observation suggests that reduced tillage systems may diminish the strength of a pre-existing hard pan, as is a key goal of CA (Troccoli *et al.* 2015). Alternatively, given that CC adoption affected BD to only a limited extent, it is quite possible that a longer time period is required to see more change as Blanco-Canqui *et al.* (2011) observed in similar pedological conditions. The complexity of the effect of CC on BD as the present study revealed in its 2020 contrasting results from before and after the main cropping season. In fact, seasonal BD changes reported in the literature are generally linked first to meteorological and biological factors (Hu *et al.* 2012) and secondarily to the time interval after tillage (Ellert and Bettany 1995; Wendt and Hauser 2013).

Penetration resistance results confirmed some BD trends. They showed lower average values when associated to wide differences in tillage intensity (i.e., ploughing *vs* no-tillage). These results agreed with some authors (Trevini *et al.* 2013) and disagreed with others (Parihar *et al.* 2016; Singh *et al.* 2016; Blanco-Canqui and Ruis 2020). It is worth noting that MT resulted as the tillage with the highest PR values, which contrasted with data obtained in similar pedological conditions, such as Sharratt *et al.* (2012). As for BD, inconsistent CC results were also found for PR. In general, WW seemed to mitigate soil strengt, while TR had either a negligible or negative effect on soil strength. In this case, the positive effects of Graminaceous CC on soil physical parameters were expected as they have often been reported (Diacono *et al.* 2019). On the contrary, the inconsistent results associated with TR were unexpected. In this moment, it is important to recall that taproot species, as the tillage radish, were originally introduced and adopted as cover crops for their beneficial effects on soil physical qualities, and in particular, soil compaction alleviation (Toom *et al.* 2019; Wittwer and van der Heijden 2020).

The inconsistent results of CC on BD and PR may stem from some methodological issues as well. One such issue is that the sampling area on which the measures were taken was limited to 39 $cm^2$ for BD and 2 $cm^2$ for PR, whereas the effect from the apparatus of a taproot cover crop could only be observed on a larger scale. Another factor may be the various values that authors have suggested as being the PR threshold (de Moraes *et al.* 2014). It can be hypothesised that under real field conditions, roots can circumvent harder zones if biopores are present. In NT in particular, the high presence of earthworms and the pores left by CC roots—possibly even weed roots—could permit subsequent crop root penetration into the soil, as observed by Crotty and Stoate (2019), despite a high average PR resistance (Hirth *et al.* 2005).

The analyses of Ks and S highlighted enhanced water infiltration under no-tillage management. Moreover, the effects seemed stronger during the second survey. These results seemed in contrast with BD and PR evidences obtained in the same period. In fact, since high BD and PR are usually linked to lower soil porosity, expected Ks values should have been lower than observed. However, contrasting results on the effects of reduced tillage on Ks can also be found in the literature (Strudley *et al.* 2008; Blanco-Canqui and Ruis 2020; Castellini *et al.* 2020). Indeed, some studies (e.g., Lipiec *et al.*, 2006; Pagliai *et al.*, 2004) found how the presence of biopores from root decomposition and earthworm activity might alleviate soil compaction by promoting preferential flow through macropores. On the other hand, others (e.g., Kahlon *et al.*, 2013; Vogeler *et al.*, 2009) suggested the loss of macroporosity under no-tillage may not sustain water infiltration. The result contrasts such as those observed across the different soil coverings may be influenced by length of the monitoring period, length of the transition period, and/or issues of scale. A marginal effect that faded during the main cropping season reported amongst the different CC has also been reported by Wagger and Denton (1989). It likely relates to the limited potential of CC to promote well-developed pore networks. Seasonal variability could also have affected soil properties and mask CC effects. Effects from the length of the transition period after conversion from conventional to CA have yet to be fully characterized, although increased soil strength is often observed in the short term. Kay and Vanden Bygaart (2002) have identified three distinct phases following CA adoption: 1) short-term phase (months): soil compaction and fragmentation is expected from tillage absence and traffic load; 2) medium-term phase (years): greater biological activity (e.g., higher numbers of earthworms) promotes the formation of vertically-oriented bio-macropores, which in turn, alleviates soil strength; 3) extended-term phase (decades): different distributions of soil organic matter stabilize soil structure and fulfil ecosystem servicing needs. In addition, sampling size may also have caused an effect; for example, CC could exert an effect observable only on a large area (e.g., sub-metric scale), even though most soil analyses (e.g., bulk density) are performed at smaller scales (e.g., centimetre-scale) (Piccoli *et al.* 2019).

In this study, the presence of a significant BD-PR regression only in the 0.5-2.5 MPa and 1.33-1.80 g $cm^{-3}$ ranges may suggest that in lower density soil profiles (i.e., BD<1.8 g $cm^{-3}$ and PR<2.5 MPa), soil structure dynamics might be governed by a centimetre scale due to a homogeneous pore network. On the contrary, higher density (e.g., BD>1.8 g $cm^{-3}$ and PR>2.5 MPa) soils might be characterized by low anisotropic porosity, in which the presence/absence of few macropores (e.g., cracks, biopores) may rule structure dynamics and soil functions in the form of water infiltration and/or gas exchanges (Piccoli *et al.* 2017b, 2019). The inconsistent results seen in no-tillage systems probably were caused by a scale issue as well. Indeed, NT evidenced soil compaction and satisfactory water infiltration simultaneously, likely due to the presence of vertically-oriented biomacropores and greater pore connectivity (Piccoli *et al.* 2017a). Consequently, both CC and NT systems are likely to produce more heterogeneous soil structure as compared with tilled soils.

## 5 Conclusions

After the first three years from conversion to CA, some effects on soil begins to be evident. From the farmer's point of view, crop yields can be satisfactory even during the transition phase, but, particularly with NT, the system appears to be more susceptible to adverse meteorological conditions.

This study proved that during the transition period from conventional to conservation agriculture some compaction issues can be linked to no-tillage when monitoring is performed with traditional small-scale physical methods (e.g., bulk density, penetration resistance) due to a high soil structure heterogeneity. Therefore, the use of larger-scale measurement, such as the double ring infiltrometer, might be preferable in no-tillage managements to overcome the inherent problems of higher spatial variability at the micro scale and to consider soil function as a whole. The fibrous root apparatus of *Poaceae* species seems a promising cover crop to enhance soil physical qualities in the no-tillage systems of Northeast Italy, even in the short term. Moreover, Graminaceous, such as winter wheat, are common cash crops in this study area and their agronomic management (e.g., sowing) is easily implemented by farmers. However, the longer period required for taproot cover crop (e.g., tillage radish) to exploit its ecosystem services, and no-till systems alike, fully requires their evaluation at a larger scale. One of the future challenges that the agronomic community will face is the termination of cover crops, especially in light of pesticide reduction, and/or the selection of winter-killed species to meet the sustainable development goals of the 2030 Agenda.

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# Chapter 3

# Reduced tillage systems and cover crop: sustainability evaluation through a multivariate analysis approach

# 1 Introduction

Conservation agriculture (CA) is defined as the combination of three principles: minimum soil disturbance, permanent soil organic cover and species diversification (FAO 2017). It could be considered an evolution of the conservation tillage, firstly introduced in USA in the 1930s, to contrast dust bowls (Hobbs *et al.* 2008). Apart the reduction of wind and water erosion, which were the main soil issues in the USA at that time, CA should reduce management costs, and provide several other ecosystem services: namely improving soil physical and chemical properties, soil organic carbon (SOC) and biodiversity, even if some of these effects are less clear (Baveye *et al.* 2011; Palm *et al.* 2014). In any case, CA has been more and more adopted all over the world, raising to 12.5% of the global cropland in 2016. Besides this, it is not widespread in Europe, where only in the 5% of total cropland is managed with CA (Kassam *et al.* 2019). Limited adoption was reported in Italy, where it is implemented in no more than 300,000 ha. The main issues during the conversion from conventional to CA are related to the transition time, when the positive effects of CA are not occurring, while the farmers face two main problems: reduction of crop yield and the need of new machinery equipment. According to Troccoli *et al.*, (2015), these two drawbacks, together with the management costs of the permanent soil covering, could need an economic support, that encourages the farmers to afford the conversion to CA.

Different experiments reported the occurrence of a long conversion time from conventional management to CA, in the Italian agroecosystem: in an experiment involving 20 farms in the Po valley, Perego *et al.* (2019) reported many CA positive effects where this management practice was implemented for a long time, concluding that the transition time depends also on farmer's knowledge and skills. Differently, in an experiment involving three farms in northern-eastern Italy, Camarotto *et al.* (2020) suggested that a transition time is needed to stabilize SOC content and other soil properties, claiming that it could take more than 6 years. Other studies conducted in the same region documented limited effects on soil porosity (Piccoli *et al.* 2017a, b), yield reduction (Piccoli *et al.* 2021) and no net effects on SOC stock (Piccoli *et al.* 2016; Longo *et al.* 2020). In regard to SOC, it is worth to note that these studies considered also the deep soil layer: although the SOC tend to increase in the surface soil layer, as reported e.g. in Valkama *et al.* (2020), in the studies where the whole soil profile is considered, no significant differences were reported between conventional tillage and CA. Despite these results, other showed that CA could represents a solution to increase the energy use efficiency, reducing the $CO_2$ emissions and prevent SOC losses (Pezzuolo *et al.* 2014, 2017). Moreover, the combination of CA and precision agriculture could increase the farm sustainability by reducing the water footprint (Borsato *et al.* 2018) and to further reduce the SOC losses and $CO_2$ emissions (Cillis *et al.* 2018).

In several field trials conducted in central and southern Italy, contrasting results were obtained. After six years of conversion time, high yield and yield quality were obtained in durum wheat by Calzarano *et al.* (2018), together with soil amelioration (Pagnani *et al.* 2019). This yield increase, in comparison to conventional agriculture, was reported also by Vastola *et al.* (2017), but only in the dry years, when on average there is a yield reduction: for this reason, they suggest to refund farmers for the ecosystem services provided by CA, thereby compensating yield losses. Similarly, in a 20-years-long experiment, Ruisi *et al.* (2014) obtained the highest yield with CA, during dry years, but no significant differences were observed when enough water was available. They also highlighted that CA could reduce yield quality, in their case durum wheat protein content. Massaccesi *et al.* (2020) and Piazza *et al.* (2020) rather reported multiple positive effects at soil level, in terms of aggregate stability, microbial community, SOC content and biodiversity, but with a significant reduction of crop yields. Positive effects on soil structure, SOC and earthworms were reported also by Stagnari *et al.* (2020); moreover, they observed lower $CO_2$ emissions in CA.

Different experiments led to different conclusion, but the ecosystem services and the transition time are two topical issues in several articles. The first are relevant for policy makers and for the environmental objectives, thus they justify CA economical supports, as is the case of greening payment provided for in Common Agricultural Policy in the EU (European Commission 2017). On the other side, yield losses and other negative effect must be limited or compensate, particularly during transition time, that seems too long to be economically sustainable. These considerations reinforce the need to address an agronomical protocol for CA, that reduces transition time and transition time's negative effect, while maximizing the ecosystem services. On one side, this protocol would be adapted and adjusted to each local area, but a homogeneous assessment criterion is needed for an early evaluation of the management practices.

The definition of an index could be a viable solution to determine and compare the impact of different management strategies. A methodology to calculate a soil quality index was defined in Masto *et al.* (2007, 2008). The procedure consists in the selection of a number of indicators, which are surveyed and normalized with linear or non-linear scoring functions. The objective of this normalization is to associate higher score to the observation reporting better performances. The multivariate analysis, by which determine the indicators and the indicators weighting factor was then described by Andrews *et al.* (2002a, b). This method was adapted and applied on different studies to evaluate: long-term practices (Masto *et al.* 2007, 2008), the combination of different crop rotations with different residues management (Armenise *et al.* 2013; Kumar *et al.* 2021), or different tillage practices (Raiesi and Kabiri 2016).

### 1.2 Objective of the work

In this work, we analysed the results of a three-year conversion time experiment, conducted in Veneto region between 2018 and 2021. The objective was to determine which and how a series of environmental indicators were affected by the combinations of tillage and cover crop. Thus, a sustainability index was calculated to compare different treatment combinations, as a function of the selected indicators variability. The no-tillage system was compared to conventional tillage and minimum tillage. These three treatments were combined with two different cover crops: tillage radish (*Raphanus raphanistrum sativus* L. *var. longipinnatus*) that is proved to have positive effects on soil physical properties, due to its taproot (Toom *et al.* 2019; Wittwer and van der Heijden 2020); and, in contrast, winter wheat (*Triticum aestivum* L.), that has a fibrous root system.

## 2 Materials and methods

The experiment took place in the Lucio Toniolo Experimental Farm, located in Legnaro, PD (NE Italy, 45° 21 N; 11° 58 E; 6 m a.s.l.). The climate is sub-humid, with temperatures between -1.5°C on average in January, and 27.2°C on average in July. Rainfalls reaches 850 mm annually, and with a reference evapotranspiration of 945 mm, that exceeds rainfalls from April to September. The highest rainfalls are reported in June (100 m) and in October (90 mm); winter is the driest season with average rainfalls of 55 mm. The water table is shallow, ranging from 0.5 to 2 m depth, with the lowest values recorded in summer.

The trial begun in spring 2018, and it was designed as a split plot, with two replicates. An area of 2 ha was divided in 18 plots of 1,111 $m^2$. The soil is a Fluvi-Calcaric Cambisol (FAO-UNESCO 2008), with a silty loam texture. The tillage treatments were randomized in the main plots: conventional tillage (CT), consisting in 30 cm depth ploughing and then harrowing (15 cm); minimum tillage (MT), where only the 15 cm of soil were tilled with a harrow; and no tillage (NT), characterized by sod seeding. Within each main plot, three winter soil coverings were randomized: tillage radish (TR – *Raphanus raphanistrum sativus*L.), winter wheat (WW – *Triticum aestivum* L.) and bare soil (BS) where no soil cover was present, but the residues of the previous year crop. Cover crops were seeded on the main crop residues in autumn 2018 and 2019. The main crop was always maize (*Zea mays* L.).

## 2.1 Field surveys

To monitor the evolution of environmental conditions, a total of 11 environmental parameters were measured. Namely, 1) aggregate stability (Agg), 2) bulk density (BD), 3) soil organic carbon (C org) and 4) total nitrogen (N tot), 5) gravimetric water content (GWC) and 6) penetration resistance (PR), 7) saturated hydraulic conductivity (Ks), 8) earthworm density (EW), 9) mineral nitrogen (N min), 10) pH and 11) cash crop yield (Y). All these parameters were measured two times: the first immediately after treatment combination adoption (T0) and then at the end of the three-year period (T1).

The Agg was determined on soil aggregates in the 0.2 - 2 cm fraction, sampled in the 0 - 20 cm layer. A continuous value of aggregate stability was determined with the Slakes application (Fajardo *et al.* 2016; Flynn *et al.* 2020). The analysis was performed on three aggregates randomly selected from each sample. The application provides a dimensionless slaking index (SI) with a value ≥ 0, as result of the analysis. It is calculated as the ratio between the initial dry aggregate area ($At_0$) and the final area (At), after 10 minutes dipping. The index is calculated with the following equation:

$$SI = \frac{A_t - A_{t0}}{A_{t0}} \qquad (1)$$

The higher SI is, the lower is soil aggregate stability. A value <3 represents high aggregate stability, between 3 and 7 indicates moderate stability, while, when the index results in values higher than 7, the aggregates have a low stability

The BD was measured in the 0-30 cm soil profile with the core method as described in Grossman and Reinsch, (2002). In the studied soil, a BD value of 1.55 g $cm^{-3}$ is considered a limiting condition to plant roots growth (USDA NRCS 1996).

The C org and N tot contents were measured in the shallow layer (0-30 cm). The soil was air dried and sieved at 0.5 mm; subsequently, SOC and TN were determined with the flash combustion method using the CNS Elemental analyser (Vario Max; Analysensysteme GmbH, Langenselbold, Germany). Inorganic carbon was removed in advance with an acid pre-treatment.

GWC was measured in the 0-20 cm soil layer while PR, measured for the 0-80 cm layer, was averaged over the same layer as GWC. Four sampling areas were selected in each plot. In each sampling area, a disturbed soil core was collected, weighted and oven-dried at 105°C to determine the GWC, while 4 repeated measures of PR were performed with the Penetrologger (Eijkelkamp, Netherland). A PR value above 2.5 MPa was considered a limiting factor to plant root growth (Groenevelt *et al.* 2001).

The double ring infiltrometer method was applied to determine the saturated hydraulic conductivity (Ks) (Parr and Bertrand 1960). The diameter of the two rings was determined according to Lai and Ren, (Lai and Ren 2007), the inner ring had a diameter ≥ 40 cm to measure both the row and the interrow area in the tillage radish plots. The water within the inner ring was kept between two levels: the operator measured the time taken by the water to reach the lower level from the highest one, then further water was added to reach again the higher level. This operation was replicated until the infiltration rate was constant. The water in the external ring was kept at an average value between the two levels of the inner ring. The data were analysed by fitting Philip's equations (Philip 1957) with the Solver Microsoft Excel add-in.

$$i(t) = S \times t^{1/2} + At \quad (2)$$

$$v(t) = \frac{S \times t^{-1/2}}{2} + A \quad (3)$$

Where i(t) and v(t) are respectively the water infiltration (m) and infiltration rate (m $sec^{-1}$) expressed in function of the time, S and A are two parameters calculated with the Excel Solver add-in, by minimizing the square difference between the predicted and the observed i(t) and v(t). The saturated hydraulic conductivity (Ks) was calculated as:

$$K_s = \frac{A}{m} \quad (4)$$

With m as a constant equal to 2/3.

The EW was measured with a mustard extraction, as described by Valckx *et al.* (2011). The measure consists in the earthworm extraction from the surface of soil, using water suspended mustard in a 25×25 cm frame (Valckx *et al.* 2011). The number of extracted earthworms was firstly used to determine a soil quality scoring (Shepherd and Janssen, 2000). According with this scoring, a density below 4 earthworms received the minimum score (poor condition), a result between 4 and 8 a medium score (moderate condition), and the best score was given to density above 8 earthworms extracted (good condition). Then, the earthworm count was compared amongst the different treatment combinations.

N min was estimated in the 0-20 cm soil layer, it consists in the measure of the concentration of ammonium, nitrite and nitrated, through a KCl extraction followed by an analysis of photometry as described by García-Robledo *et al.* (García-Robledo *et al.* 2014).

To measure soil pH a soil sample was collected in the 0-20 cm soil layer, air dried, mixed, sieved at 0.5 mm. Afterwards, the pH was measured in 1 M KCl solution (1 : 2.5 solid/ liquid ratio) (Van Reeuwijk 1986).

At the end of the cropping season, four biomass samples were collected within each subplot, to determine maize grain yield (Y) at 27% grain moisture. After the harvest, a grain sample was air dried at 105°C until constant weight to determine the dry mass weight. Yield was expressed in kg of dry grain per hectare.

## 2.2 Data analysis

First, a mixed-effects model was applied to test the main effect of tillage, covering and their interaction on all i-th variables for each monitored year. All effects given above were treated as fixed effects, block as random. Post hoc pairwise comparisons of least squares means were performed using the Tukey method to adjust for multiple comparisons.

A methodology to calculate a soil quality index was defined in Masto *et al.* (Masto *et al.* 2007, 2008). The procedure consists in the selection of a set of indicators, which are surveyed and normalized with linear or non-linear scoring functions. The objective of this normalization is to associate higher score to the observation reporting better performances. The multivariate analysis, by which determine the indicators and the indicators weighting factor was then described by Andrews *et al.* (Andrews *et al.* 2002a, b). This method was adapted and applied on different studies to evaluate: long-term practices (Masto *et al.* 2007, 2008), the combination of different crop rotations with different residues management (Armenise *et al.* 2013; Kumar *et al.* 2021), or different tillage practices (Raiesi and Kabiri 2016).

Afterwards, each sampling data were normalized with a linear scoring function, as described in (Masto *et al.* 2008) applying equations (5), (6) and (7)

$$S = \frac{x_{ij} - x_{i\,min}}{x_{i\,max} - x_{i\,min}} \quad (5)$$

$$S = -\frac{x_{ij} - x_{i\,max}}{x_{i\,max} - x_{i\,min}} \quad (6)$$

$$S = \frac{|x_{ij} - 7|}{|x_i - 7|_{max} - |x_i - 7|_{min}} \quad (7)$$

With $x_{i\,max}$ as the maximum value measured during the i parameter survey, and $x_{i\,min}$ the smallest. The S value ranges between 0, corresponding to the minimum value observed in the i parameter and 1, for the maximum. Equation (5) was used for "More is better" scoring function, here C org, GWC, Ks, EW, N min, N tot and Y. Differently, AS, BD and PR were scored with equation (6), according with the "Less is better" approach. Finally, equation (7) was used for pH scoring. The implementation of these three equations led to score higher values in the treatment combination with the best impact on the parameters.

The Relative Sustainability Index (RSI) was calculated as the sum of the observed parameter score, weighted with principal component analysis weighting factors (PWs). These factors were calculated with the procedure described in Masto (Masto *et al.* 2008), by selecting principal components (PCs) explaining at least 10% of the variability. Within each of these PCs, loaded factor, with values >|0.2| were selected and their correlation were measured as in Andrews *et al.* 2002b in case of correlation (r>|0.8|), only the factor with the highest loading factor was used for RSI calculation, together with all the other uncorrelated highly loaded factors. The percentage of variation explained by each PC provided the PW. RSI was calculated with equation (8):

$$RSI = \sum_{i=1}^{n} PW_i \times S_i \quad (8)$$

To normalize RSI, it was divided by the highest RSI value obtained. A total of 36 RSIs were calculated: one per each treatment combination replication in the T0 survey and another in the T1.

RSI differences amongst the treatment combinations were tested with mixed models. All possible first and second order interactions between factors were tested, and the model with the smallest AIC (Akaike's Information Criterion) was selected (Schabenberger and Pierce 2001). Post hoc pairwise comparisons of least squares means were performed using the Tukey method to adjust for multiple comparisons. Microsoft Excel 2016, ClustVis (Metsalu and Vilo 2015) and SAS (SAS Institute Inc. Cary, NC, USA) version 5.1 were used for statistical analysis.

## 3 Results

The mixed model results are showed in table 1, while table 2 reports the average observation values in T0 and T1, from which the RSIs were calculated.

Agg, GWC and pH observations in T0 resulted significantly higher than in T1. Specifically, the stability index lowered, on average, from 4.50 in T0 to 3.19 in T1, resulting in higher aggregate stability. Considering both T0 and T1, Agg ranged between 0.3 and 6.1, resulting in moderate to high aggregate stability. In T0, all the sample resulted in a moderate aggregate stability (>3), except one collected in the MT-BS treatment combination, where a value of 2.9 was observed. During the T1 survey the 44% of the observations resulted below the threshold of 3 (high aggregate stability), the lowest aggregate stability index values were found under reduced tillage systems (i.e., NT and MT). GWC was strictly related to the pedoclimatic conditions in the sampling dates, being in the 20-25% and 12-22% range in 2019 and 2020, respectively. In addition, the CC treatments had significant effects on GWC. In fact, TR and WW reported values of 18.3% and 20.3% respectively, the BS treatment reported an intermediate value. Even if pH reduced significantly from T0 to T1, it ranged between non-critical values, on average 7.36 in T0 and 7.05 in T1.

N tot, PR and Ks increased significantly from T0 to T1. N tot grew from 0.88‰ in T0 to 1.01‰ in T1, moreover, it maintained a modest variability within each survey (the coefficient of variations was 0.26 in T0 and 0.13 in T1). PR test varied between an average value of 0.70 MPa in T0 to 1.34 MPa in T1. Even if in the second survey PR was significantly higher and with a higher variability, all the observations resulted below the 2.5 MPa threshold. Considering the different tillage systems, CT reported a value of 0.88 MPa, which resulted significantly lower than 1.18 MPa., observed under NT. MT reported an intermediate value. Lastly, Ks showed a +158% increase from T0 ($3.4 \times 10^{-5}$ m $sec^{-1}$) to T1 ($8.7\times 10^{-5}$ m $sec^{-1}$). This parameter resulted also significantly impacted by different tillage intensity, NT reported on average $1.05 \times 10^{-4}$ m $sec^{-1}$ Ks value, while CT resulted in a value of $3.58 \times 10^{-5}$ m $sec^{-1}$. Again, MT showed an intermediate result.

BD and EW resulted affected by the time and tillage combination. In particular, BD showed limited differences among the treatments, but it reported low values in CT during the first survey (1.39 g $cm^{-3}$, on average), while the other survey resulted on average 1.45 g $cm^{-3}$. All the measures resulted below the 1.55 g $cm^{-3}$ threshold. EW variability was higher, ranging between 0 and 20 (table 2). The higher difference was observed during the second survey in the NT treatment, which reported an average value of 13.17, resulting significantly higher than the data observed in CT (3.00).

C org reported an average value of 0.83%, resulting in modest variability within and between the surveys. Similarly, Y reported an average value of 10.00 Mg $ha^{-1}$ and N min resulted on average 24.54 ppm, with no significant differences among the different treatment combination in the different survey.

**Table 1. Comparison of p values among the linear mixed-effect models analysis of observed parameters (Agg: aggregate stability, BD: bulk density, C org: soil organic carbon, N tot: soil total nitrogen, GWC: gravimetric water content, PR: penetration resistance, Ks: saturated hydraulic conductivity, EW: earthworm density, N min: mineral nitrogen, Y: yield). Symbology: ▪ p<0.1; * p<0.05; ** p<0.01; *** p<0.001.**

| | Agg | | BD | | C org | N tot | | GWC | | PR | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Time | 0.001 | ** | 0.155 | | 0.715 | 0.052 | ▪ | <0.001 | *** | <0.001 | *** |
| Tillage | 0.111 | | 0.663 | | 0.633 | 0.188 | | 0.255 | | 0.004 | ** |
| CC | 0.831 | | 0.529 | | 0.990 | 0.870 | | 0.030 | * | 0.635 | |
| Time×Till | 0.928 | | 0.043 | * | 0.350 | 0.192 | | 0.443 | | 0.334 | |
| Time×CC | 0.507 | | 0.469 | | 0.768 | 0.545 | | 0.808 | | 0.815 | |
| Till×CC | 0.227 | | 0.672 | | 0.778 | 0.766 | | 0.677 | | 0.724 | |
| Time×Till×CC | 0.112 | | 0.536 | | 0.882 | 0.566 | | 0.915 | | 0.877 | |

| | Ks | | EW | | N min | pH | | Y |
|---|---|---|---|---|---|---|---|---|
| Time | 0.034 | * | 0.389 | | 0.451 | <0.001 | *** | 0.840 |
| Tillage | 0.046 | * | 0.126 | | 0.906 | 0.159 | | 0.904 |
| CC | 0.187 | | 0.104 | | 0.615 | 0.982 | | 0.680 |
| Time×Till | 0.390 | | 0.006 | ** | 0.169 | 0.551 | | 0.760 |
| Time×CC | 0.564 | | 0.199 | | 0.589 | 0.612 | | 0.378 |
| Till×CC | 0.252 | | 0.161 | | 0.343 | 0.867 | | 0.648 |
| Time×Till×CC | 0.680 | | 0.796 | | 0.501 | 0.970 | | 0.589 |

**Table 2. Summary of parameters values, in T0 and T1 surveys (Agg: aggregate stability, SI: stability index, BD: bulk density, C org: soil organic carbon, N tot: soil total nitrogen, GWC: gravimetric water content, PR: penetration resistance, Ks: saturated hydraulic conductivity, EW: earthworm density, N min: mineral nitrogen).**

| Survey | Parameter | Unit of measure | Minimum | Maximum | Average value | Standard deviation | Coefficient of variation |
|---|---|---|---|---|---|---|---|
| T0 | Agg | SI | 2.90 | 6.10 | 4.50 | 0.92 | 0.20 |
| | BD | g $cm^{-3}$ | 1.32 | 1.54 | 1.43 | 0.05 | 0.04 |
| | C org | % | 0.64 | 1.07 | 0.83 | 0.12 | 0.14 |
| | N tot | ‰ | 0.08 | 1.09 | 0.88 | 0.23 | 0.26 |
| | GWC | % | 20 | 25 | 23 | 1 | 0.06 |
| | PR | MPa | 0.46 | 1.05 | 0.70 | 0.14 | 0.21 |
| | Ks | m $sec^{-1}$ | $6.7 \times 10^{-6}$ | $1.7 \times 10^{-4}$ | $3.4 \times 10^{-5}$ | $3.9 \times 10^{-5}$ | 1.15 |
| | EW | n $m^{-2}$ | 0.00 | 16.00 | 6.17 | 4.69 | 0.76 |
| | N min | ppm | 12.85 | 46.90 | 22.97 | 8.91 | 0.39 |
| | pH | -- | 7.22 | 7.49 | 7.36 | 0.06 | 0.008 |
| | Yield | Mg $ha^{-1}$ | 5.41 | 12.36 | 9.96 | 1.62 | 0.16 |
| T1 | Agg | SI | 0.30 | 5.20 | 3.19 | 1.25 | 0.39 |
| | BD | g $cm^{-3}$ | 1.36 | 1.56 | 1.46 | 0.06 | 0.04 |
| | C org | % | 0.63 | 1.01 | 0.82 | 0.11 | 0.13 |
| | N tot | ‰ | 0.74 | 1.21 | 1.01 | 0.13 | 0.13 |
| | GWC | % | 12 | 22 | 16 | 2 | 0.13 |
| | PR | MPa | 0.96 | 1.96 | 1.34 | 0.25 | 0.19 |
| | Ks | m $sec^{-1}$ | $8.2 \times 10^{-6}$ | $3.6 \times 10^{-4}$ | $8.7 \times 10^{-5}$ | $1.0 \times 10^{-4}$ | 1.16 |
| | EW | n $m^{-2}$ | 0.00 | 20.00 | 7.44 | 6.21 | 0.83 |
| | N min | ppm | 6.49 | 53.41 | 26.11 | 14.37 | 0.55 |
| | pH | -- | 6.93 | 7.22 | 7.05 | 0.08 | 0.01 |
| | Yield | Mg $ha^{-1}$ | 9.28 | 11.09 | 10.04 | 0.55 | 0.05 |

The values presented in Table 2 were normalized. The average of each treatment combination is presented in Figures 1 (biochemical parameters) and 2 (physical parameters. Because of this normalization, higher values are associated to the parameter improvement, and wider areas to an overall sustainability increment.

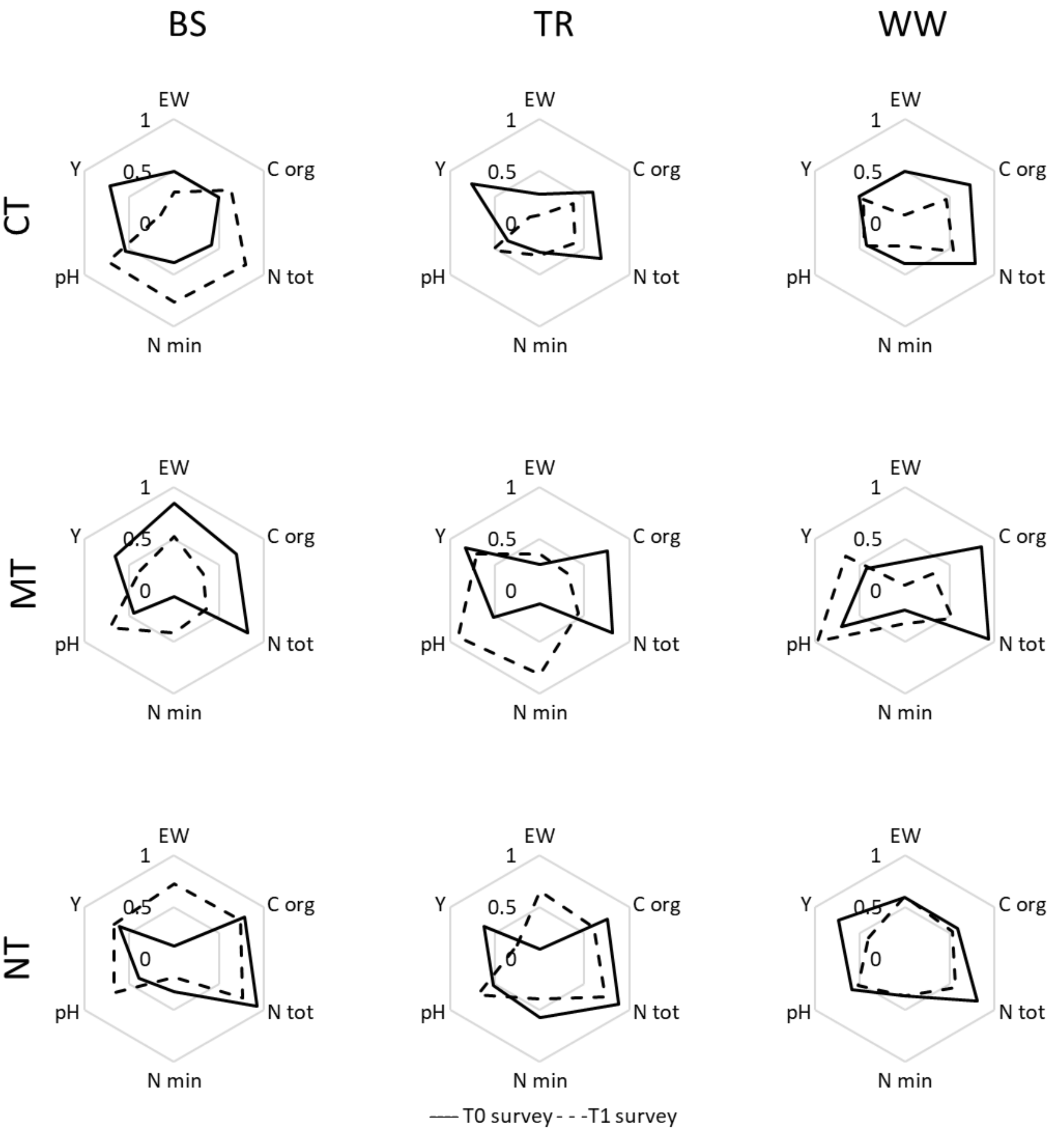


**Figure 1. Biochemical parameter scores, average values in treatment combinations in T0 and T1 surveys (C org: soil organic carbon, N tot: soil total nitrogen, EW: earthworm density, N min: mineral nitrogen, Y: yield, BS: bare soil, TR: tillage radish, WW: winter wheat, CT: conventional tillage, MT: minimum tillage, NT: no tillage).**

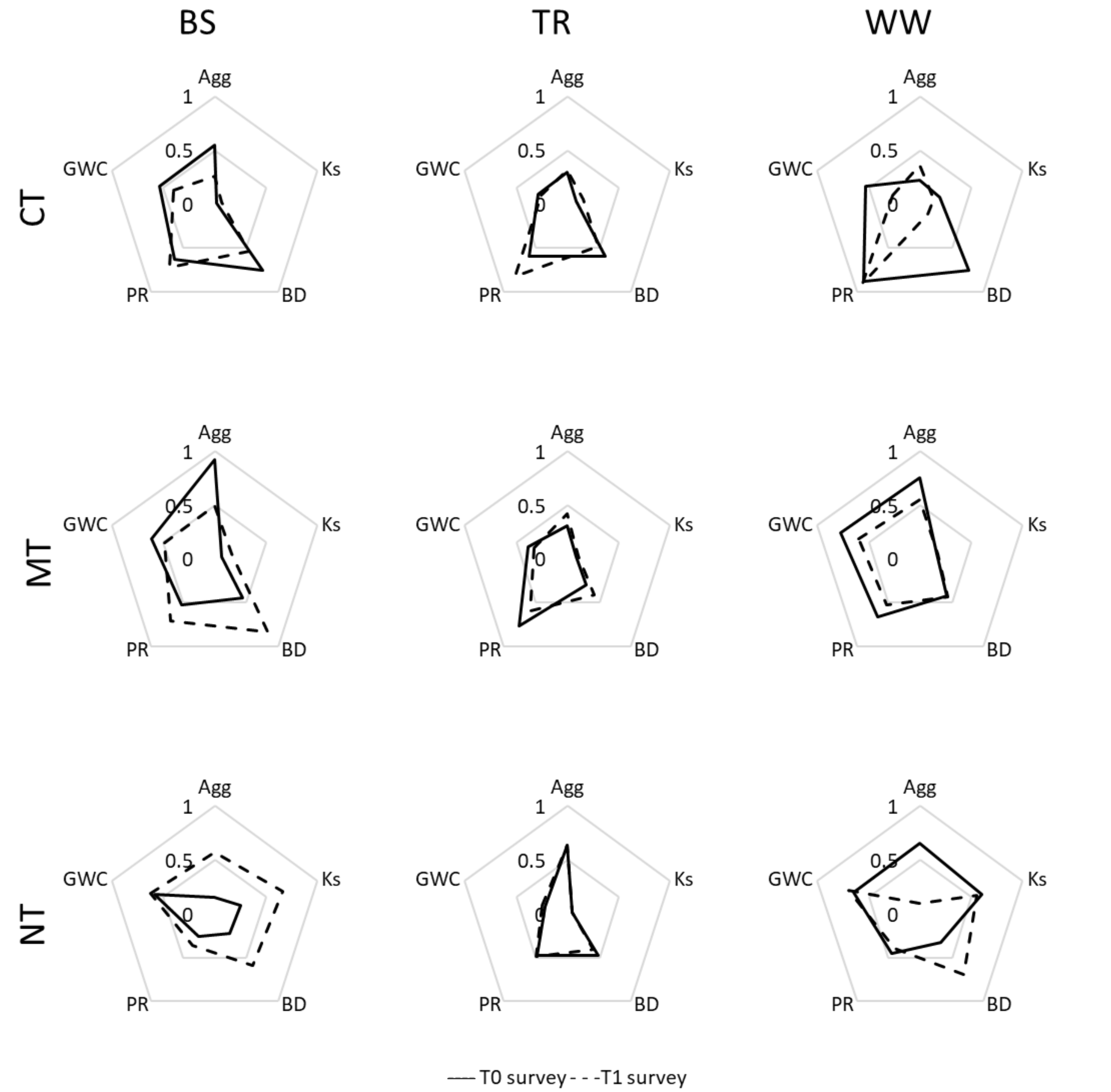


**Figure 2. Physical parameter scores, average values in treatment combinations in T0 and T1 surveys (Agg: aggregate stability, BD: bulk density, GWC: gravimetric water content, PR: penetration resistance, Ks: saturated hydraulic conductivity, BS: bare soil, TR: tillage radish, WW: winter wheat, CT: conventional tillage, MT: minimum tillage, NT: no tillage).**

Figures 1 and 2 show both deep differences between the treatment combinations and the two years. The correlation matrix between the parameter is shown in table 3. The highest correlation was observed between C org and N tot (r=0.924), which was the only value above the |0.8| threshold. Consequently, only one of these two parameters was included in the RSI, namely the one with the highest weight in the PCA.

**Table 3 Correlation among the parameters. Bold values are considered highly correlated (r>0.8), according with Andrews *et al* (2002b). (Agg: aggregate stability, BD: bulk density, C org: soil organic carbon, N tot: soil total nitrogen, GWC: gravimetric water content, PR: penetration resistance, Ks: saturated hydraulic conductivity, EW: earthworm density, N min: mineral nitrogen, Y: yield).**

| | Agg | BD | C org | EW | GWC | Ks | N min | N tot | pH | PR | Y |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Agg | 1 | | | | | | | | | | |
| BD | -0.009 | 1 | | | | | | | | | |
| C org | -0.131 | 0.187 | 1 | | | | | | | | |
| EW | -0.274 | 0.234 | 0.033 | 1 | | | | | | | |
| GWC | -0.152 | 0.299 | 0.340 | 0.175 | 1 | | | | | | |
| Ks | 0.032 | 0.007 | 0.256 | 0.310 | 0.344 | 1 | | | | | |
| N min | 0.385 | 0.091 | -0.134 | -0.153 | -0.150 | -0.037 | 1 | | | | |
| N tot | -0.165 | 0.100 | **0.924** | 0.038 | 0.328 | 0.293 | -0.022 | 1 | | | |
| pH | -0.091 | -0.038 | 0.000 | -0.069 | 0.115 | 0.100 | 0.182 | -0.086 | 1 | | |
| PR | 0.038 | 0.130 | -0.203 | -0.271 | 0.012 | -0.469 | -0.154 | -0.284 | -0.039 | 1 | |
| Y | -0.043 | -0.119 | 0.009 | 0.104 | -0.182 | 0.144 | -0.088 | 0.024 | -0.037 | -0.332 | 1 |

The PCA results are shown in table 4. These data were implemented to determine the parameters weights. Namely, the parameters weight was equivalent to the variation explained by the PC selected.

**Table 4. Results of principal component analysis, under different treatment combination in different years. Bold factor loadings are considered highly weighted, bold-underlined factors were included in the RSI calculation (Agg: aggregate stability, BD: bulk density, C org: soil organic carbon, N tot: soil total nitrogen, GWC: gravimetric water content, PR: penetration resistance, Ks: saturated hydraulic conductivity, EW: earthworm density, N min: mineral nitrogen, Y: yield).**

| Principal components | PC-1 | PC-2 | PC-3 | PC-4 | PC-5 |
|---|---|---|---|---|---|
| Variation | 0.241 | 0.149 | 0.135 | 0.117 | 0.100 |
| Cumulative variation | 0.241 | 0.389 | 0.525 | 0.642 | 0.742 |
| Agg | 0.196 | -0.134 | **<u>0.536</u>** | -0.062 | 0.347 |
| BD | 0.153 | **<u>-0.355</u>** | -0.072 | 0.420 | -0.417 |
| C org | **0.498** | -0.168 | -0.222 | -0.334 | 0.012 |
| EW | **<u>0.248</u>** | 0.131 | 0.403 | 0.441 | -0.263 |
| GWC | **<u>0.355</u>** | -0.326 | -0.018 | 0.302 | 0.129 |
| Ks | **<u>0.378</u>** | 0.304 | -0.057 | 0.277 | 0.041 |
| N min | -0.124 | 0.176 | **<u>-0.601</u>** | 0.265 | -0.070 |
| N tot | **<u>0.504</u>** | -0.097 | -0.244 | -0.357 | -0.018 |
| pH | 0.010 | 0.046 | -0.169 | **<u>0.338</u>** | 0.777 |
| PR | -0.291 | **<u>-0.563</u>** | 0.073 | -0.056 | 0.020 |
| Y | 0.079 | **<u>0.502</u>** | 0.196 | -0.174 | -0.098 |

Under PC-1 the parameter selected were N tot, GWC, Ks and EW. Since C org and N tot resulted highly correlated, only N tot was included in the RSI, considering that it reported a higher weight under PC-1. Then in PC-2 highly weighted parameters were BD, PR, and Y. A limited correlation was reported amongst these parameters, then all of them were included in the RSI. Finally, Agg and N min were selected in PC-3 and pH in PC-4. The resulting RSI then was expressed by equation (9):

$$RSI = \frac{0.135Agg + 0.149BD + 0.241EW + 0.241GWC + 0.241Ks + 0.135N\min + 0.241N\,tot + 0.117pH + 0.149PR + 0.149Y}{1.247} \quad (9)$$

The weighting factor of each parameter is equal to the explained variability of the PC selected for that specific factor, namely 0.241 for PC-1, 0.149 for PC-2, 0.135 for PC-3 and 0.117 for PC-4. The sum of the weighted parameter was divided by 1.247, that was the highest sum of weighted factor reported amongst all the observation (reported in NT-WW combination in block 1, for the T0 survey), so it was adopted to normalize the RSI. The lowest in CT-TR block 2 in T1, with a value of 0.358. The smallest AIC on the linear mixed model on the RSI was obtained when Intercept, Tillage and Covering where tested as fixed factor and block as random factor. The p values of these mixed models are summarized in table 5.

**Table 5. Linear mixed model analysis of RSI output. Symbology: ▪ p<0.1; * p<0.05; *** p<0.001**

| | T0 | | | | | T1 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Effect | F | Df | Df.res | Pr(>F) | | F | Df | Df.res | Pr(>F) | |
| Intercept | 75.81 | 1 | 10.7 | <0.001 | *** | 81.27 | 1 | 6.1 | <0.001 | *** |
| Tillage | 0.20 | 2 | 12 | 0.823 | | 5.57 | 2 | 12 | 0.019 | * |
| Soil covering | 2.48 | 2 | 12 | 0.125 | | 2.88 | 2 | 12 | 0.095 | ▪ |

Inconsistent differences were reported during the first survey, while in T1 a significant (p<0.05) tillage effect was reported. According with Tukey post hoc test, NT reported an RSI equal to 0.752, significantly higher than the one reported in CT (RSI=0.529) and with MT reporting an intermediate value (RSI=0.663).

Figure 3 shows the average RSI of the different tillage system, with the contribution of each parameter. The parameters with the highest impact on RSI are GWC and N tot with an average contribution to RSI equal to 0.091 and 0.131, respectively. During the T1 survey the highest score in these two parameters were observed in NT, where GWC reported a value of 0.102 and N tot of 0.133. Despite Ks and EW reported, on average, a limited contribution to RSI, these two parameters showed high variability amongst treatments. Particularly, during the T1 survey Ks resulted in a value of 0.082 in NT, three times the values observed in MT (0.028), or CT (0.023). EW, similarly, reported a value of 0.127 in NT, twice as much as in MT (0.060) and fourfold the value observed in CT (0.029).

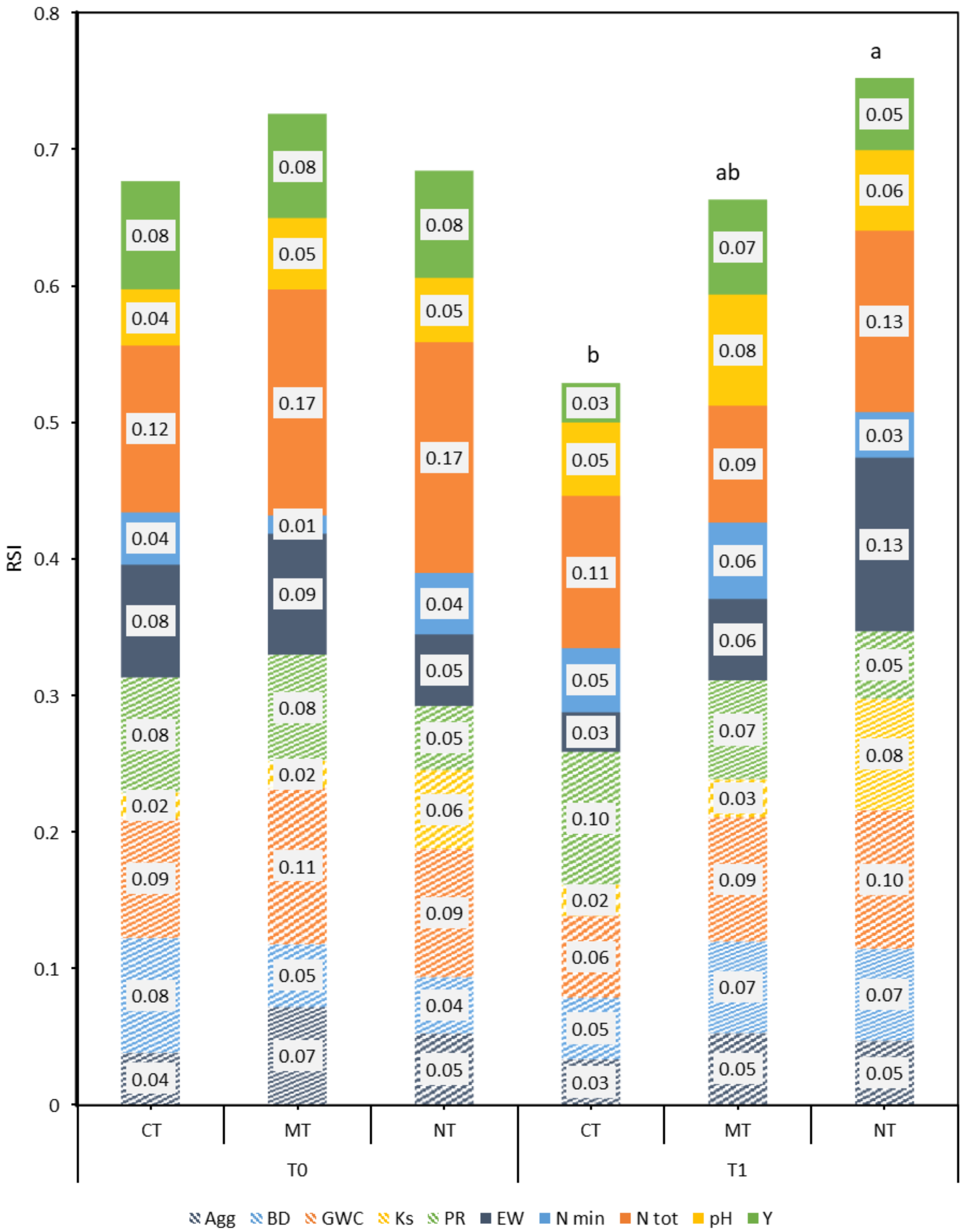


**Figure 3. Average RSI and parameters contribution for different tillage in different years. The letters indicate significant tillage effect according to the Tukey test (p<0.05). Agg: aggregate stability, BD: bulk density, GWC: gravimetric water content, Ks: saturated hydraulic conductivity, PR: penetration resistance, EW: earthworm density, N min: mineral nitrogen, N tot: soil total nitrogen, Y: yield, CT: conventional tillage, MT: minimum tillage, NT: no tillage. Different letters represent significant differences of the global treatment RSI according to the post-hoc Tukey test (p<0.05).**

No clear effect was instead observed for the soil covering treatment, in both years where no statistical differences were recorded. The minimum RSI was always reached in TR in both years (0.604 in 2019 and 0.583 in 2020) while higher values were recorded in WW in 2019 and in BS in 2020 (figure 4).

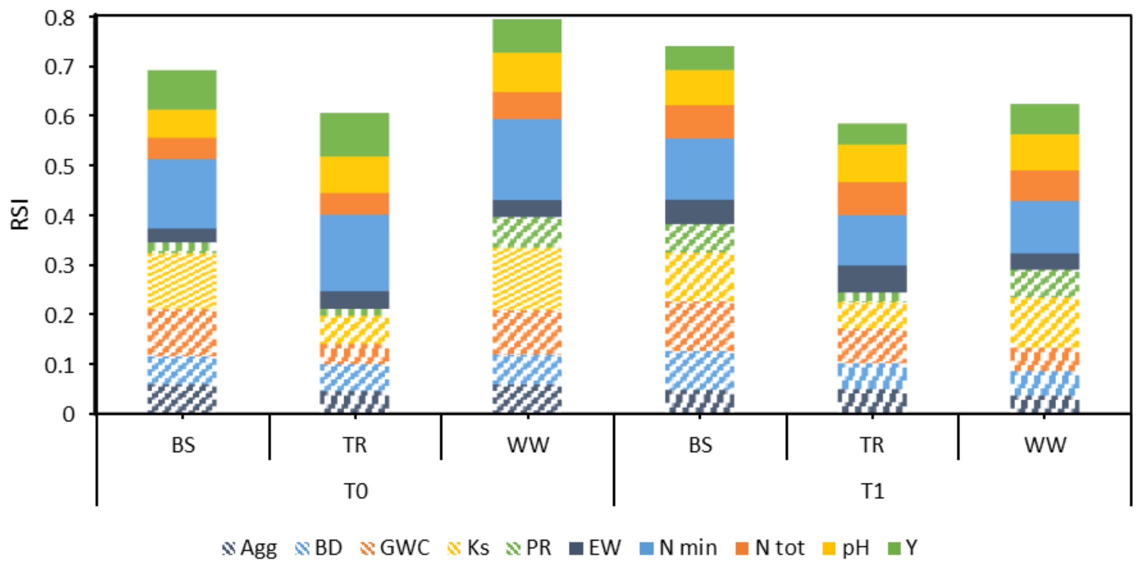


**Figure 4. Average RSI values for different soil covering in different years. BS: bare soil, TR: tillage radish, WW: winter wheat.**

## 4 Discussion

Eight out of eleven parameters revealed a significant variation amongst the observations. This suggests that the soil system was under an evolution, irrespectively from the treatments. On one side, these differences could be related to the environmental condition dynamic during T0 and T1. Nevertheless, GWC was affected by the different CC. The effects of CC on water cycle resulted controversial: in fact, different authors suggested alternatively the positive effects of CC on water balance and water availability (García-González *et al.* 2018), other findings reported soil water reduction for the subsequent crop, after CC termination (Krstić *et al.* 2018). In this study, higher GWC was observed under WW, while TR reported the lowest value.

The different tillage systems seemed to have a stronger impact on the parameter, particularly on BD, PR, Ks and EW. The first two reported better values under CT: this seems in line with previous evidence, according to which the application of reduced tillage systems could increase soil strength and bulk density, especially in the first years (Blanco-Canqui and Ruis 2018). Considering that BD and PR were measured in the 0-20 cm soil layer, a reduction of this value under CT was an expected tillage effect. This could also be related with the instrument resolution, as the measure of penetration resistance could be negatively impacted by the high spatial variability under reduced tillage system (Piccoli *et al.* 2019). Moreover, BD was almost always below the threshold of 1.55 g cm$^{-3}$. Under these values plant root growths should not be limited in silty loam soils (USDA NRCS 1996). Similarly, PR variates between 0.46 and 1.96 MPa: an increment of this parameter in this range should have a limited effect, since the threshold is usually set at 2.5 MPa (Groenevelt *et al.* 2001). Finally, the soil where the experiment was performed is characterized by structural inertia in response to management changes (Piccoli *et al.* 2017, 2020; Camarotto *et al.* 2018).

Even if BD and PR values worsened under reduced tillage systems, soil function was maintained, as Ks resulted higher in NT (+193% if compared to CT in T1). This could be related with the higher EW observed under this management. In fact, earthworm bio-macropores could significantly contribute to soil function and in particular air and water permeability, even in compacted soils. Earthworms can improve soil structure (Bertrand *et al.* 2015) and hydraulic properties (van Schaik *et al.* 2014) by burrowing and casting. The positive effects of NT on EW confirmed previous studies evidence (Crotty and Stoate 2019; Perego *et al.* 2019; Stagnari *et al.* 2020).

The effects on EW were underlined also after the RSI computation, from which EW resulted an effect with high weight within the index (11% on average) and a high variability between the different treatment. Other parameters which highly impacted on RSI composition were N tot, GWC and PR (17%, 14% and 11% respectively, on average), which together accounted for more than 50%, on average, of the RSI. The highest impact on RSI variability was driven by Ks, N min and Y, together with EW. In absolute terms, Ks, N min and Y had an impact lower than 10% on the RSI, but their variation coefficients were the highest, ranging between 0.67 (Ks) and 0.31 (Y). Thus, these could be considered the best indicators for the evolution of soil under conversion from conventional tillage to CA. In particular, RSI results suggested that Ks and EW are two sustainability indicators, and they are positively affected by NT.

The final RSI score showed a positive effect of NT in respect to conventional tillage, even in the short time. MT resulted mid-way between CT and NT, with the amelioration of some physical parameters, but a lesser improvement on biological traits, namely on EW. Similarly, Issaka *et al.* (2019) observations, which described that both minimum and no-tillage as sustainable techniques, considering the nutrient cycles. Differently from what observed by other authors (Perego *et al.* 2019; Camarotto *et al.* 2020; Piccoli *et al.* 2020), a clear negative transition time effect was not detected during this three years experiment.

The magnitude of reduced tillage systems effects, together with the sampling methods could have masked a possible CC effect. In fact, limited differences were reported among the different soil covering managements. CC effects could require longer conversion time, or different sampling methodology (Wagger and Denton 1989). Moreover, it is worth to note that BS was partially covered by spontaneous plants, such as weeds, which could have an impact on soil properties, like what expected with CCs. In fact, it has been observed that spontaneous plant CC can provide some ecosystem services (Herencia 2017; Torres *et al.* 2018; Carpio *et al.* 2019; Guzmán *et al.* 2019a, b). Moreover, the presence of plant residues could positively impact on microbial diversity (Li *et al.* 2020), which can be considered an environmental sustainability indicator (Anyanzwa *et al.* 2008).

From another point of view, the modest effect of TR could be related to the sampling timing: in fact, most of the expected TR benefits (related with the porosity and pore connectivity enhancement) are delivered only when the taproots are degraded. Nevertheless, other authors observed how tillage radish might be effective also at the short time (Toom *et al.* 2019; Wittwer and van der Heijden 2020), a longer timespan period might be necessary to exploit the benefits of TR in terms of soil properties (Camarotto *et al.* 2020). The bio-tillage effect, which was expected from TR, as suggested by Zhang and Peng (2021), could be masked by earthworm activity in NT treatments, irrespectively from the presence of TR. The high EW values observed under NT could had performed this bio-tillage effect, which, according to the authors could replace the conventional tillage.

Then, even if the WW fibrous root apparatus should have a limited impact on soil structure, many Poaceae CC improved overall system sustainability (Diacono *et al.* 2019; Ciaccia *et al.* 2019), and aggregate stability (García-González *et al.*

2018; Guzmán *et al.* 2019b; Domagała-Świątkiewicz *et al.* 2019), or nutrient cycles (Wen *et al.* 2019; Fiorini *et al.* 2020; Norberg and Aronsson 2020). The combination of grass CC and reduced tillage systems proved to positively affect environmental sustainability, fostering biodiversity (Reeves 1997) and soil organic carbon (Calegari *et al.* 2008).

In conclusion, to correctly evaluate the CA effect, and especially on the soil system, a holistic approach should be preferred to consider both the effects on crop production, but also on soil physics, considering different soil function at different scales (Vogel *et al.* 2021)..

## 5 Conclusion

The short term effects of the different tillage intensities, combined with the different management of soil covering gave limited result; nevertheless a multivariate analysis of selected sustainability indicators was adopted to calculate a relative sustainability index (RSI). This revealed a positive effect of reduced tillage systems management, and in particular NT. This positive result could be the effect of an increased soil fauna activity, which could contribute to soil structure improvement. In consequence, NT seemed to have an impact on soil physics and soil habitability, resulting in a significant higher RSI value.

The effect of CC was limited, but WW reported the best results in the short time, with a tendency to have higher RSI values. Collectively, the combination of NT and WW can be considered the most promising in terms of sustainability improvement. Longer term experiment could better evaluate the effects of these management on some parameters such as soil organic carbon, which have a wide impact on sustainability, but vary little in the short term.

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# Chapter 4

# Tillage radish and white mustard seeding date: a case study in Northern Italy

## 1 Introduction

The use of Cover Crop (CC) has become one of the pivotal strategies to improve farm sustainability (Schipanski *et al.* 2014; Adetunji *et al.* 2020). It consists in the cultivation of selected crop species between two main cash crops, the CC biomass is left on the field, or buried before the subsequent cycle, with a wide range of positive effects, from microbial biodiversity to yield improvement (Garland *et al.* 2021). Different CC species with different characteristics can deliver different ecosystem services; nevertheless, all CC need to: 1) develop and establish rapidly, 2) quickly cover the soil, 3) produce a sufficient dry matter, 4) do not host pest or pathogens, 5) be easily terminated at the end of their cycle, 6) be economically viable (Reeves 1994). Some of these points could seem in contradiction: on one side a rapid development is crucial, on the other CC must be cost-effective, as they do not contribute directly to profitability. Moreover, at the end of the cycle if not properly terminated, the CC could disseminate and trigger a weed infestation.

Each CC species or CC mixture could be selected for a target effect, such as weeds or pests suppression (Cherr *et al.* 2006; Büchi *et al.* 2020), their effects on nutrient cycling (Thorup-Kristensen *et al.* 2003; Wittwer *et al.* 2017), the improvement of biodiversity and other environmental services (Crotty and Stoate 2019) or their effects on soil properties (Blanco-Canqui *et al.* 2015).

Tillage radish (*Raphanus raphanistrum sativus,* L. – TR) is a Brassicaceous species, which was selected as a cover crop to improve soil porosity and soil structure, because of its root apparatus. In fact, TR is a deep-rooted CC, with a wide and deep taproot (Williams and Weil 2004). TR efficacy in improving soil property, and possibly reducing the need of tillage operation was reported by Toom *et al.* (2019a) and Wittwer and van der Heijden (2020). Moreover, it proved to contrast weed development, with two mechanisms: competition and allelopathy (Schappert *et al.* 2018; Sturm *et al.* 2018; Ranaldo *et al.* 2019; Schappert *et al.* 2019b; Schmidt *et al.* 2019). Nevertheless, if on one side this species seems effective in contrasting weed, other authors reported inconsistent impacts (Salonen and Ketoja 2019). Another effect reported was the nutrient cycling improvement (Zhao *et al.* 2020; Norberg and Aronsson 2020). Finally, TR termination should be facilitated by its frost sensitivity, in fact it proved to be winter killed if it reaches an adequate development, in a sufficient cold winter (Büchi *et al.* 2020). TR seems a promising CC in a conservation agriculture system, as it should contrast soil compaction, which is often expected in the first years of conservation agriculture management (Palm *et al.* 2014; Piccoli *et al.* 2020). However, information on TR adaptability to Northern Italy agroecosystem is still limited, and site-specific studies are needed to assess its effectiveness in provide the expected ecosystem services and its performances.

Another Brassicaceous species, which can be adopted as a CC is the white mustard (*Sinapis alba,* L. – WM). Differently from TR, WM is more common and well adapted to Northern Italy environment, proving to be frost sensitive if sufficiently developed. Even if its root apparatus results less developed in comparison with TR, it proved to reduce soil compaction (Ren *et al.* 2019). Moreover, it can develop rapidly and cover the soil with its canopy, providing positive effect in contrasting runoff and erosion (Torres *et al.* 2018). As for TR, the combination of competition and allelopathy could suppress weeds (Schappert *et al.* 2018, 2019a), in addition WM could contrasts also some pathogens (Berlanas *et al.* 2018; Kadziene *et al.* 2020) and interact with pesticides (Cassigneul *et al.* 2018). Finally, WM could improve soil organic matter and nutrient cycling (Torres *et al.* 2018; Plaza-Bonilla *et al.* 2018; Toom *et al.* 2019b).

Both these CC are seeded in autumn, establish rapidly, and should be frost killed during winter. One of the crucial stages in both TR and WM cultivation is the seeding date (Darby *et al.* 2016). In fact, their development could impact on their frost sensitivity, moreover most of the expected benefits are not provided in case of limited growth; on the other side, if these CC reach the reproductive stage, they will become weeds.

Since TR should provide a soil structure amelioration, in optimal conditions it should affect positively parameters such as bulk density or soil hydraulic conductivity. The aim of this work is to determine the optimal seeding date for TR and WM, by monitoring their development and frost sensitivity. Then, two soil physical parameters are compared, to assess which are the effects on soil porosity and pore connections of the different seeding dates combined with the different species.

## 2 Materials and methods

The experiment took place in the Lucio Toniolo Experimental Farm, located in Legnaro, PD (NE Italy, 45° 21 N; 11° 58 E; 6 m a.s.l.). The sub-humid climate reports temperatures between -1.5°C on average in January, and 27.2°C on average in July. Rainfalls reaches 850 mm annually and are exceeded by the evapotranspiration from April to September. Over year the reference evapotranspiration is of 945 mm. The rainfall ranges between 100 mm in June and 90 mm in October (the highest) and 55 mm in winter, that is the driest season. The water table ranges from 0.5 to 2 m depth, with the lowest values recorded in summer.

The two-year trial begun in July 2019, and it was designed as a complete randomized block design, with four replicates, for a total of 36 plots of 9 $m^2$. The soil is a Fluvi-Calcaric Cambisol (FAO-UNESCO, 2008), with a silty loam texture. Two winter cover crop (CC) were compared: tillage radish (*Raphanus raphanistrum sativus* L. – TR) and white mustard (*Sinapis alba* L. – WM); in combination with four seeding date: July, August, September, October. This management was repeated both in 2019 and 2020. During the summer the plots were set aside, and only weed management was performed, with one Glyphosate application. This was aimed to reduce the interaction between CC and main crop, in order to measure the effects on soil properties. Moreover, the trials occupied different portion of the field in different portion of the year, this would have imposed the adoption of different main crop with different interactions with the CC. An untreated plot (bare soil – BS) was selected in each block. Here, during the two years the soil was kept bare, and only weed management was performed. Each seeding date was identified by the seeding month (July, August, September, October).

To simulate field conditions, the CC were manually seeded after a minimum tillage. The adopted density was 22 plants $m^{-2}$. Six rows were drilled in each plot, the distance between the rows was 45 cm.

In both years a series of measures were taken to monitor the cover crop development. Two indexes were measured: Normalized Difference Vegetation Index (NDVI) and Leaf Area Index (LAI). NDVI was measured with the Crop Circle (Holland Scientific, Nebraska, USA). The LAI was measured only when applicable (i.e., when the crop was sufficiently developed), with the SunScan (Delta-T Devices, United Kingdom). To monitor soil temperature, five soil temperature-moisture sensors were installed, two in a TR plot, the first at 5 cm depth, the second at 30 cm, two at the same depths in a WM plot, finally one at 5 cm depth in a BS plot.

In spring, visual assessment (VA) was performed to assess the CC development, the weed infestation, and the frost sensitivity. To do so, the different plots were compared and classified according to the CC development and weed infestation. Main phenological traits (PT), such as plant height, number of leaves, presence of flowers, were registered during the surveys. Moreover, during the second year the soil covering was measured with the app Canopeo (Patrignani and Ochsner 2015).

In spring 2021 two soil physical measures were performed. Firstly, saturated hydraulic conductivity (Ks) was measured in the plot on an area of 1,300 $cm^2$, with the double ring infiltrometer method, in each plot (Philip 1957). Then soil bulk density (BD) was measured in the 0-10 cm layer with the core method (Grossman and Reinsch 2002). To better assess the effect of the roots on BD, the measure was performed on an area of 177 $cm^2$. This area was considered an appropriate sampling size to better evaluate CC effects, that are often underestimate on smaller scales, as observed by Piccoli *et al.* (2019)

The seeding and sampling dates are summarized in table 1

**Table 1. Seeding dates and surveys. TR7: tillage radish seeded in July, TR8: tillage radish seeded in August, TR9: tillage radish seeded in September, TR10: tillage radish seeded in October, WM7: white mustard seeded in July, WM8: white mustard seeded in August, WM9: white mustard seeded in September, WM10: white mustard seeded in October. PT: phenological traits, VA: visual assessment, Ks: saturated hydraulic conductivity, BD: bulk density.**

| Dates 2019-20 | Seeding dates | Surveys | Dates 2020-21 | Seeding dates | Surveys |
|---|---|---|---|---|---|
| 18/07 | WM7, TR7 | | 20/07 | WM7, TR7 | |
| 19/08 | WM8, TR8 | | 22/08 | WM8, TR8 | |
| 21/08 | | PT, NDVI | 22/09 | WM9, TR9 | |
| 16/09 | WM9, TR9 | PT, NDVI, LAI | 28/09 | | PT, NDVI, LAI |
| 01/10 | | PT, NDVI, LAI | 06/10 | | PT, NDVI, LAI |
| 08/10 | | PT, NDVI, LAI | 22/10 | WM10, TR10 | |
| 15/10 | WM10, TR10 | | 29/10 | | PT, NDVI, LAI |
| 22/10 | | PT, NDVI, LAI | 09/11 | | PT, NDVI, LAI |
| 07/11 | | PT, NDVI, LAI | 19/11 | | PT, NDVI, LAI |
| 13/11 | | PT, NDVI, LAI | 01/12 | | PT, NDVI, LAI |
| 20/11 | | PT, NDVI, LAI | 14/12 | | PT, NDVI, LAI |
| 03/12 | | PT, NDVI, LAI | 11/01 | | PT, NDVI, LAI |
| 10/12 | | PT, NDVI, LAI | 25/01 | | PT, NDVI |
| 17/12 | | NDVI | 09/02 | | PT, NDVI, VA |
| 14/05 | | PT, NDVI, | 05/03 | | VA |
| | | LAI, VA | 22/03 | | VA |
| | | | 20/04 | | Ks, BD |

## 2.1 Statistical analysis

A mixed-effects model was applied to test the main effects of cover crop species, seeding date, year and their interactions on November and December average NDVI and LAI values.

Other mixed-effects models were applied to test the effects on data collected with Canopeo during the 2020-2021 growing season, and on soil physical parameters.

All factors named above were treated as fixed effects, block was considered as random effect. All possible first and second order interactions between factors were tested, and the model with the smallest AIC (Akaike's Information Criterion) was selected (Schabenberger and Pierce 2001). Post hoc pairwise comparisons of least squares means were performed using the Tukey method to adjust for multiple comparisons. The correlation between NDVI, LAI and Soil covering was F-tested.

## 3 Results

Mean, maximum and minimum air temperature recorded during the two-year experiment are summarized, by month in table 2; together with the total rainfall, rainfall days and days below -3°C. Considering the meteorological differences between the two years, 1) the first growing seasons (from July to December 2019) reported higher temperature in October and November if compared with the same 2020 period. In October 2019, the average temperature resulted 18% higher than October 2020. Similarly, November 2019 mean temperature resulted 27% higher in 2019. 2) in 2019, the first days below 0°C were reported in December, the 5th, while in 2020 the 22nd of November. 3). The 2019 growing season reported higher rainfall (467 mm in 2019 vs 425 in 2020), which resulted also better distributed, while in 2020 most of rainfalls were registered in August (tenfold the value measured in August 2019) and December (+18% if compared to December 2019). 4) the average temperatures reported between December and February were almost equal (5.4°C in 2019-2020 vs 5.3°C in 2020-2021), but a temperature below -3°C was reported only in four January 2020 days; differently, the subsequent year this temperature was reached in a total of 12 days between January and February.

**Table 2. Monthly average temperature and rainfall; rainfall and frost days.**

| Year | Month | T min (°C) | Average T (°C) | T max (°C) | Total rainfall (mm) | Rainfall days | Day below -3°C |
|---|---|---|---|---|---|---|---|
| **2019** | Jun | 13.2 | 25.0 | 37.6 | 9 | 1 | |
| | Jul | 13.7 | 24.5 | 34.8 | 82.2 | 12 | |
| | Aug | 14.4 | 24.5 | 32.3 | 16.2 | 9 | |
| | Sep | 9.2 | 19.6 | 32.8 | 67.8 | 9 | |
| | Oct | 7.7 | 15.7 | 25.7 | 61.4 | 12 | |
| | Nov | 3.3 | 10.6 | 20.0 | 150 | 19 | |
| | Dec | -2.7 | 5.7 | 15.3 | 89.6 | 9 | |
| **2020** | Jan | -3.5 | 3.3 | 12.6 | 14.8 | 3 | 4 |
| | Feb | -2.9 | 7.3 | 16.7 | 4.8 | 3 | |
| | Mar | -2.5 | 9.2 | 20.9 | 59.8 | 7 | |
| | Apr | -0.5 | 13.9 | 25.8 | 24.4 | 5 | |
| | May | 8 | 18.0 | 27.2 | 30.4 | 12 | |
| | Jun | 11.3 | 21.2 | 32.8 | 142.2 | 11 | |
| | Jul | 13.4 | 23.9 | 35.7 | 43.8 | 10 | |
| | Aug | 14.7 | 24.2 | 35.2 | 175.4 | 12 | |
| | Sep | 8.1 | 20.4 | 31.8 | 19.2 | 8 | |
| | Oct | 3.5 | 13.3 | 22.7 | 66.6 | 18 | |
| | Nov | -2.6 | 8.4 | 19.8 | 14.6 | 4 | |
| | Dec | -2.1 | 5.8 | 15.0 | 105.8 | 15 | |
| **2021** | Jan | -4.7 | 3.1 | 11.8 | 71.4 | 8 | 9 |
| | Feb | -6 | 7.1 | 22.3 | 16.2 | 7 | 3 |
| | Mar | -2.7 | 8.4 | 24.6 | 4.4 | 4 | |

These differences seemed to limitedly affect soil temperature (Figure 1), that maintained the same trends in both years, with a minimum temperature of 1.16 °C reported in January 2020 at 5 cm depth. In the subsequent year, the lowest temperature reported at that depth was 1.49°C, while at 30 cm depth, the temperature remained always above 3°C degree in both years. Considering a thermal threshold of 0 °C, the growing degree days (GDD) are represented in figure 2. The differences between the seeding dates had an impact firstly on biomass development. In fact, October seeded CC development was limited, while in the other plots the biomass reached a value between 1 and 3 Mg ha$^{-1}$.

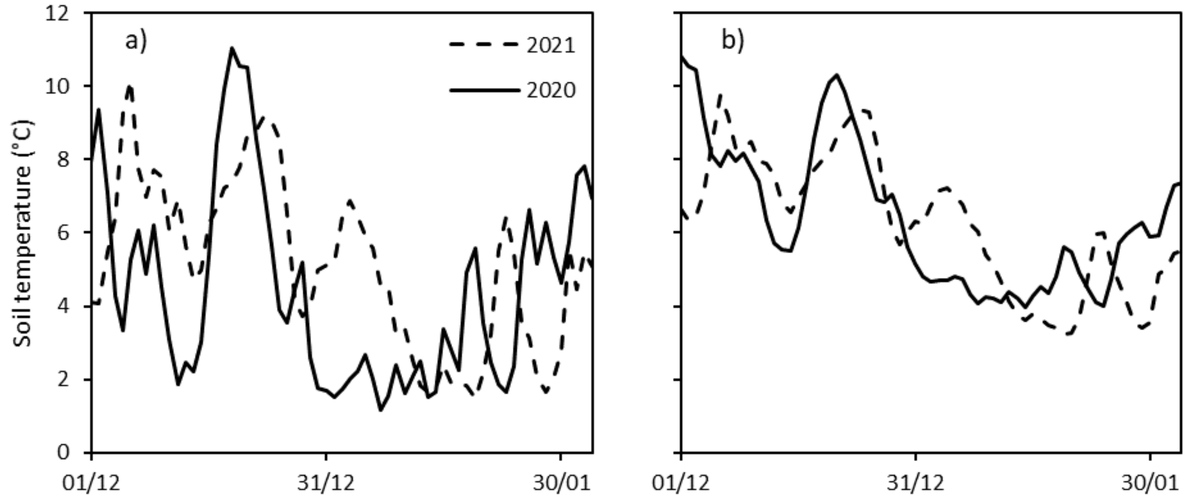


**Figure 1. Average soil temperature in 2020 and 2021 winters. a) soil temperature at 5 cm depth, b) soil temperature at 30 cm depth.**

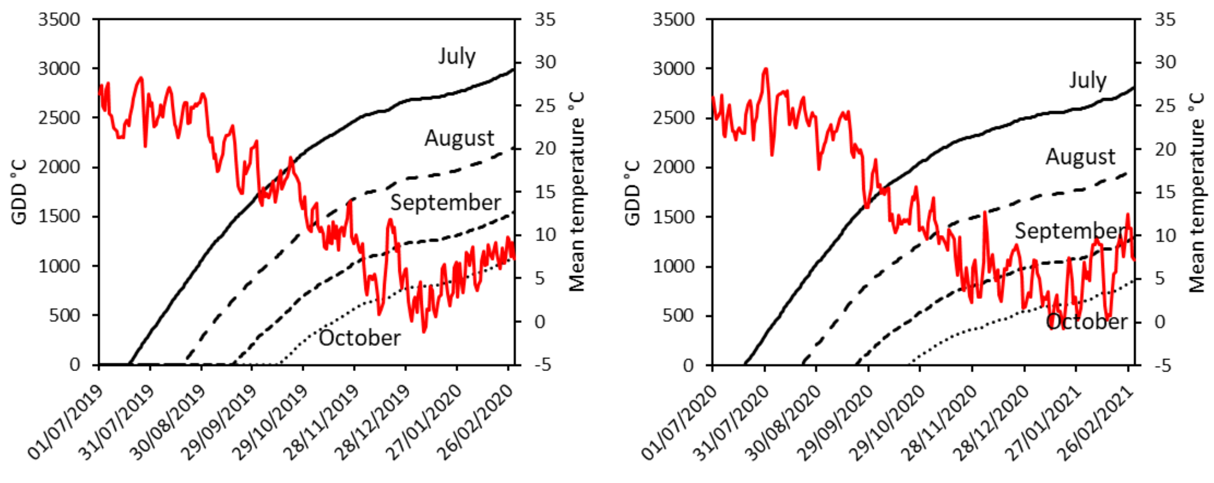


**Figure 2. Growing degrees days (black lines) and average air temperatures (red lines) in the two years**

The level of significance of each treatment and treatment combination is summarized in the subsequent table (table 3).

**Table 3. Comparison of p values among the linear mixed-effect models analysis of NDVI, LAI, soil covering, bulk density (BD) and saturated hydraulic conductivity (Ks). Effects were considered significant if p≤0.05. CC: cover crop, SM: seeding month, Y: year. Bold values represent the effect selected for the subsequent post hoc Tukey test.**

| | NDVI | | LAI | | Soil Covering | | | BD | Ks |
|---|---|---|---|---|---|---|---|---|---|
| | Nov | Dic | Nov | Dic | 19-Nov | 01-Dec | 14-Dec | | |
| Interc. | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | 0.063 |
| CC | 0.044 | 0.005 | <0.001 | 0.001 | 0.631 | 0.365 | 0.796 | ** | **0.023** |
| SM | <0.001 | <0.001 | 0.029 | 0.011 | <0.001 | <0.001 | <0.001 | **0.008** | ** |
| Year | 0.487 | 0.835 | <0.001 | 0.029 | # | # | # | # | # |
| CC*SM | **<0.001** | **0.006** | **<0.001** | **0.002** | **0.008** | **0.005** | **0.002** | ** | ** |
| CC*Y | 0.026 | 0.008 | <0.001 | 0.003 | # | # | # | # | # |
| SM*Y | <0.001 | 0.002 | <0.001 | <0.001 | # | # | # | # | # |

# Not applicable **Excluded according with AIC

Figure 3 shows the average NDVI value measured both in TR and WM, in November and December. The post hoc Tukey test divided the November measures in two classes: the first had an average NDVI value of 0.649, and counted all the treatment combinations, except TR-Jul, TR-Oct and WM-Oct which were included in the second class, with an average value of 0.422. The mean NDVI value observed in November was 0.555. this value grew to 0.567 in December. Even if this difference was limited, it was mainly driven by the October seeded plots: if compared to November results, these plots increased their NDVI on average the 24% in TR and 29% in WM. Despite this NDVI increment, which reached on average a value of 0.436 in TR-Oct and WM-Oct, these treatments remained statistically lower than the average value of the highest NDVI plots (namely TR-Aug, TR-Sep, WM-Jul, WM-Aug, WM-Sep), which scored on average a value of 0.620. WM-Jul data was collected only in 2019-2020, because the subsequent year it resulted in a crop failure in most of the blocks.

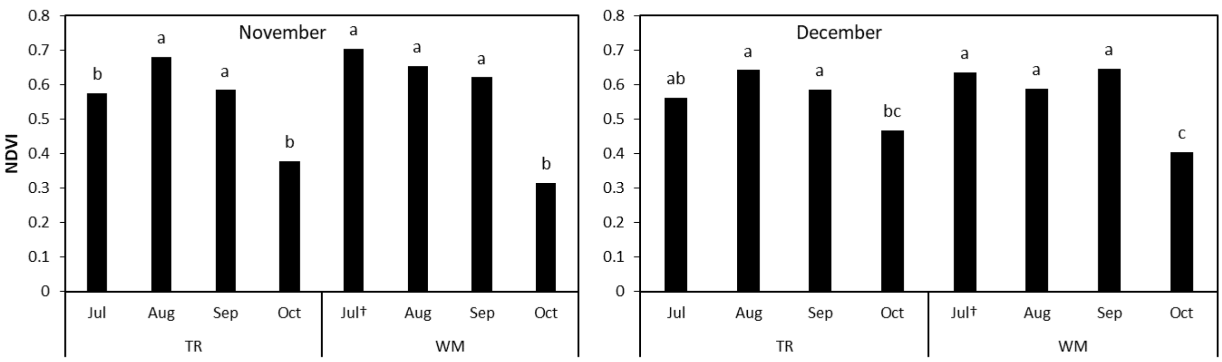


**Figure 3. Average monthly NDVI values for November and December. TR: tillage radish, WM: wite mustard. † WM-Jul was sampled only in 2019. The letters indicate significant effects of CC x seeding month according to the Tukey test (p<0.05).**

As October seeded plot development resulted limited, thus LAI was measured only in the other plots. The average LAI values are presented in figure 4. Firstly, this value tended to reduce from November (2.96 on average) to December (1.72), equal to a 42% reduction. In the first month, the highest LAI values were reported in WM-Jul, TR-Aug and TR-Sep with an average value of 3.64. December presented more homogeneous results, with the only difference reported between

WM-Jul (2.56) and TR-Jul (1.01). In fact, TR-Jul reported the lowest values also in November (2.13), while WM-Aug and WM-Sep, which resulted among the lowest in November surveys (with an average LAI value of 2.37), in December resulted in line with the other plots, reporting an average LAI of 1.68.

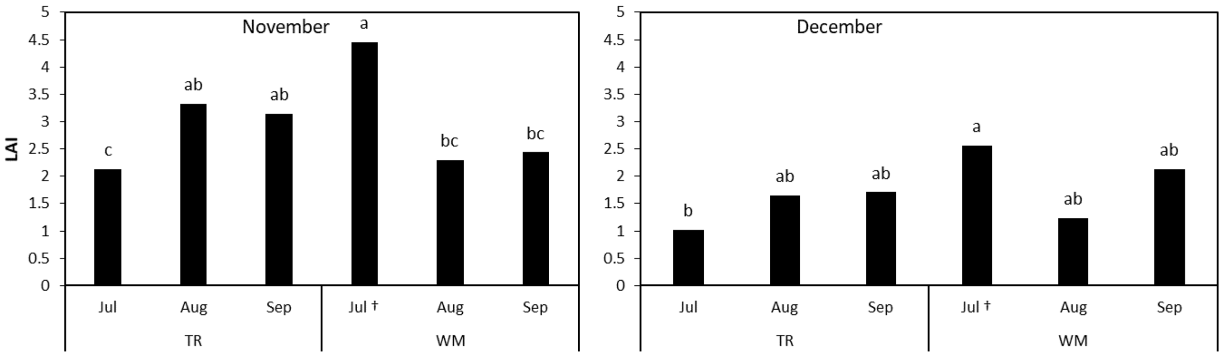


**Figure 4. Average monthly LAI values for November and December. TR: tillage radish, WM: wite mustard. † WM-Jul was sampled only in 2019. The letters indicate significant effects of CC x seeding month according to the Tukey test (p<0.05).**

In figure 5, the percentage of soil covering measured with the app Canopeo in three significant dates is presented. August-seeded plots showed high percentage of soil covering in November, the 19th. Both TR and WM showed average values above 60%. While, on the one hand, WM-Sep did not report significant differences with the two August plots, on the other, TR-Sep soil covering percentage resulted significantly lower. In December, soil covering percentage progressively reduced in TR-Aug, WM-Aug and TR-Sep. Differently, WM-Sep seemed to reach its highest soil covering value during the last survey (65%). Both TR and WM seeded in October resulted always in soil covering <20% in all the three dates, with TR showing a constant trend, while WM seemed to increase its value from 6% in November to 11% in December, the 14th.

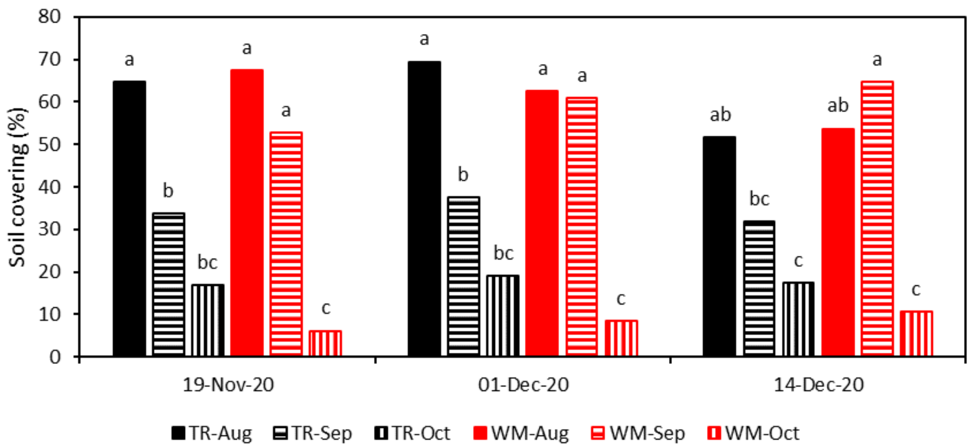


**Figure 5. Average Soil Covering percentage in three sampling dates. TR: tillage radish, WM: wite mustard. For each sampling date, the letters indicate significant effects of CC x seeding month according to the Tukey test (p<0.05).**

Bulk density, at the end of the experiment, reported a significant seeding date effect (p<0.05), irrespectively from the CC species. Figure 6 shows how the best performance on BD was reported from September and October seeded CC, with an average value of 1.27 g cm$^{-3}$, while in August the average value reached 1.34 g cm$^{-3}$ and 1.38 g cm$^{-3}$ in July. BS reported the highest BD value, on average 1.47 g cm$^{-3}$.

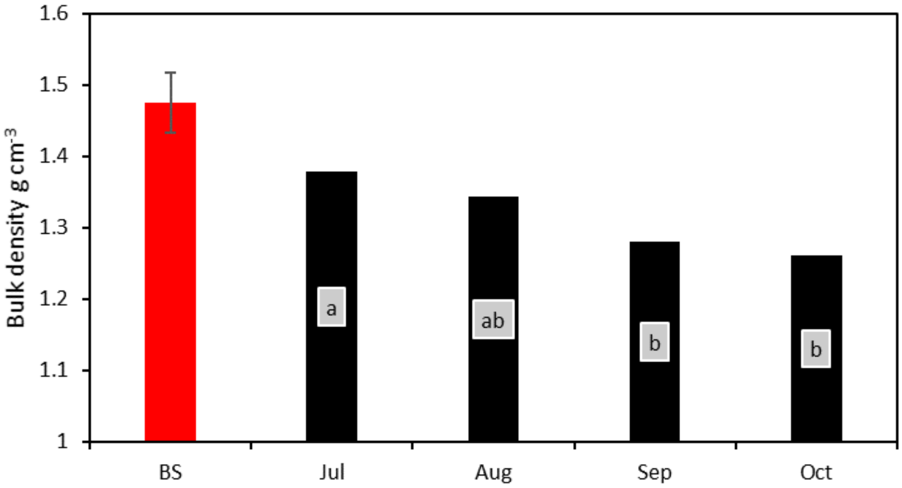


**Figure 6. Average Bulk density values, sampled at the end of the experiment. The letters indicate significant effects of seeding month according to the Tukey test ($p<0.05$).**

A net difference between CC and BS were observed on the saturated hydraulic conductivity (Figure 7). No differences were reported between the two cover crops, with on average a value of 3.7 × 10$^{-4}$ m s$^{-1}$ for TR and 2.8 × 10$^{-4}$ m s$^{-1}$ for WM. Differently, saturated conductivity measured in BS resulted significantly lower with a value of 1.4 × 10$^{-4}$ m s$^{-1}$

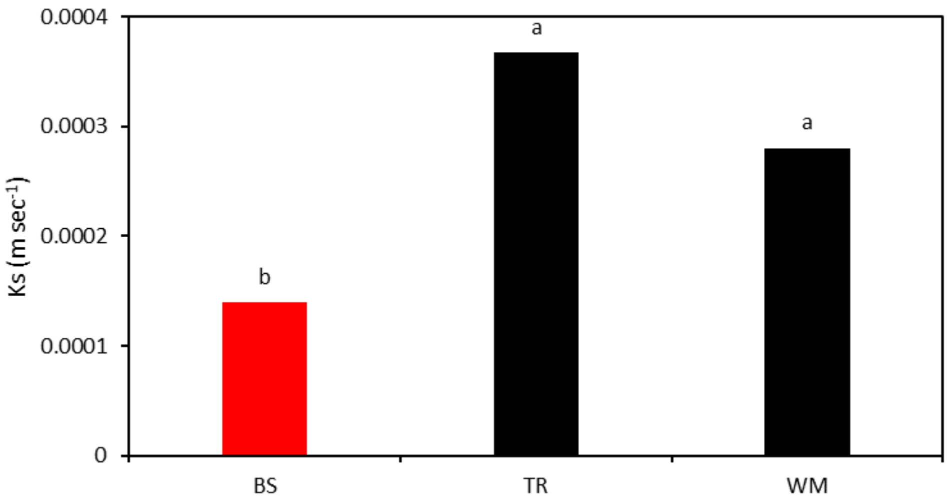


**Figure 7. Average soil saturated hydraulic conductivity (Ks) values, sampled at the end of the experiment. The letters indicate significant effects of seeding month according to the Tukey test ($p<0.05$).**

During the first year, the presence of flowers was reported in several plots, by the end of November. Namely, flowers were observed in all WM-Jul and TR-Jul plots, three out of four WM-Aug plots, one TR-Aug and one WM-Sep plot. Moreover, the presence of siliquae was observed in one of the WM-Jul plots. On the other side, before winter, the October seeded plots reported a limited development, with the issuance of 5-7 leaves for TR and 6-8 for WM. After winter, WM-

Jul, WM-Aug and WM-Sep resulted frost killed, while a vegetative restart was observed in the other treatment combinations.

The subsequent year, a reduced development was observed in most of the plot if compared with 2019-2020 season. WM-Jul resulted in a crop failure and weed infestation was observed in July plots. Again, October seeded plots development resulted limited before the winter, reaching the issuance of 2-4 leaves both in TR and WM. On the other hand, none of the treatment combinations reached the flowering stage. After winter, all plots were winter killed, except those that were seeded in October. In spring, the lower weed presence was observed in the October-seeded plots, where the CC survived and continued their growth after the winter.

## 4 Discussion

The seeding date impacted on many of the observed parameters, in line with present literature (Sturm *et al.* 2017; Toom *et al.* 2019b).

Both TR and WM showed frost sensitivity, as observed in Büchi *et al.* (2020) for TR, or in Dorsainvil *et al.* (2005) and Storr *et al.* (2020) for WM. Nevertheless, these CC survived to the winter in case of late seeding (namely, in October); moreover, TR required lower temperature, as in 2020/21 winter, which reported a total of twelve days below -3°C. In general, the second experimental year was characterized by worse environmental conditions, which resulted also in WM-Jul crop failure. CC seeded in July and August needed emergency irrigation, that represents a double threat: first, the CC management costs increase, then the irrigation could stimulate the weed infestation, nullifying the possible contrasting effects of CC (Sturm *et al.* 2018; Schappert *et al.* 2019b). In fact, CC weed control was observed under water limited condition (Schappert *et al.* 2019a). In this study, TR and WM showed a poor effect in contrasting weeds, claiming that an effective weed management require a holistic approach to be efficient (Harker and O'Donovan 2013), and targeted field experiment would better evaluate this aspect. Moreover, some literature reported also limited results in weed suppression both for TR (Salonen and Ketoja 2019) and WM (Baldivieso-Freitas *et al.* 2018).

NDVI, LAI and soil covering reported a significant correlation ($p<0.01$), the linear regression among these parameters is shown in figure 8. Despite their correlation, these indexes provide complementary information on the vegetation development. NDVI tended to maintain high values and grew from November to December, but TR-Jul, TR-Aug, WM-Jul and WM-Aug reduced their values by 2%, 6%, 10% and 10% respectively, while TR-Sep, TR-Oct, WM-Sep and WM-Oct grew by 0.3%, 24%, 4% and 28% respectively. This difference could be associated to a general deterioration of early seeded CC, due to leaves loss or leaves senescence. The NDVI increase, in October seeded CC, was expected, as October plots showed limited development, and survived to winter in both years. Differently, TR-Sep and WM-Sep seemed to continue their growth and maintain high NDVI during the first weeks below 0°C, but then they were winter-killed.

If on one side NDVI gives information on both soil covering and leaves health, on the other hand it seemed to saturate when LAI is above 1.5-2, providing only a partial information on the canopy status (possibly the information is limited to the upper leaves and the vegetative apex). This evidence is supported by Liu *et al.* (2012), which identified an effective correlation between NDVI and LAI, when LAI < 2. Moreover, LAI is measured below the CC canopy: this reduces the possibility to collect LAI data during early stages, but on the other side, it is not affected by the presence of spontaneous plants at ground level, such as weed sprouts. This spontaneous vegetation could increase the value of both NDVI and soil covering measure. In fact, from November to December the LAI decreased in all the plots by a factor between 13% in WM-Sep to 52% in TR-Jul. This information, together with NDVI results, leads to the conclusion that in December, CC in all plots started their senescence, but this process was partially mitigated in September seeded plots, where the apex and the upper leaves maintained their vitality.

From the soil covering point of view, TR-Aug, WM-Aug, and WM-Sep provided the best performance. Observing the results, WM reported a better soil covering, considering all the three dates. Particularly, WM-Sep maintained high level of soil covering even in the last survey. TR soil covering potential resulted limited. The TR early seeding seemed, on one side, to have the potential to reach good soil covering, and consequently to provide some benefits such as weed suppression; on the other side, anticipate the seeding date expose to the risk of low germination and limited CC development (Sturm *et al.* 2017).

These evidences seemed to indicated August, and even more September as the most suitable seeding dates in terms of vegetation development. In fact, a good soil covering together with a lush vegetation should provide the expected ecosystem services, such as nutrient cycling improvement (Norberg and Aronsson 2020), subsequent cash crop promotion (Bocianowski and Majchrzak 2019; Toom *et al.* 2019c) soil protection (Torres *et al.* 2018), and overall sustainability improvement (Diacono *et al.* 2019; Ciaccia *et al.* 2019).

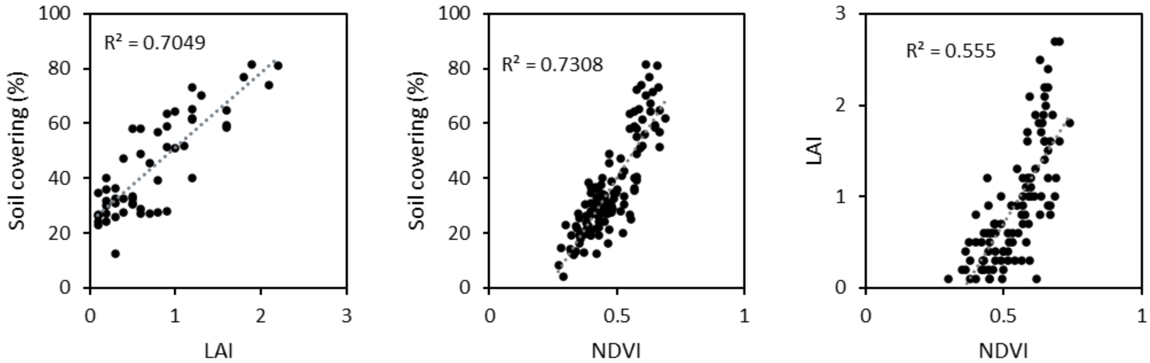


**Figure 8. Correlation between LAI and soil covering, NDVI and soil covering, NDVI and LAI**

The effects on soil physical properties resulted less pronounced, as probably a longer time is required to obtain significant results (Abdollahi *et al.* 2014). Nevertheless, the tested CC are expected to improve soil properties. Particularly, TR was selected for its deep and wide taproot that should improve soil porosity and thus soil function, as observed by Toom *et al.* (2019a) and Wittwer and van der Heijden (2020). This study results could confirm this expectation, as BD was positively affected by the CC seeding date, irrespectively of the CC species. This proved also that WM could improve soil physical quality, even in the short term, as observed in Ren *et al.* (2019); the magnitude of this effect, in the short term, resulted similar to TR results. September seeded CC provided the stronger effects of BD reduction, together with October seeded plots. The reason behind the little effect observed in early seeded CC could be related to the limited development, which was observed by the monitoring of vegetative parameter, particularly in July seeded plots. Moreover, Wagger and Denton (1989) observed that CC effects often tend to fade during the season, as their magnitude is limited and possibly masked. The observed differences could be also affected by the soil bed preparation, which could reduce shallow soil BD, with higher effect-size in the last seeded plots. Anyway, all treatment combination resulted to have a lower BD, if compared to BS. Finally, the spontaneous vegetation could affect these parameters and the presence of weed could contribute to reduce the shallow soil BD. Positive effects of spontaneous plant on soil physical parameters were observed by (Torres *et al.* 2018; Guzmán *et al.* 2019a, b).

Similarly to what observed on BD, positive CC effect were reported also on Ks. The double ring infiltrometer allowed to study a large area, which include the biopores left after root decomposition, which are thought to promote preferential flows (Pagliai *et al.* 2004; Lipiec *et al.* 2006). These biopores could significantly affect pore connection, thus increase soil structure, soil function and Ks, with limited effect on BD (Hirth *et al.* 2005). Finally, Ks resulted significantly higher in both TR and WM, if compared to BS. Between the two CC, TR tended to report a higher Ks value, which could support the expectations behind this CC.

# 5 Conclusion

The vegetation indexes, the visual assessment, the soil monitoring and the agronomic considerations seemed to indicate September as the best month for both TR and WM seeding. This option ensured a decent development, without the need of irrigation. In case of sufficient cold temperature during winter, both TR and WM showed frost sensitivity, if seeded within September. Their effects on soil physical parameter resulted modestly related to seeding date or species, but the most promising combination for these parameters seemed TR seeded in September. On the other side, WM-Sep ensured the canopy development that should provide soil protection and ecosystem services.

This plot experiment was intended to monitor the development and the effect on soil of the two CC, in the short time, to assess the potential of TR and WM in local conditions. Larger field experiment could better evaluate their potentiality, when integrated in the agroecosystem.

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# Chapter 5

# General conclusions

In contrast to previous studies indicating an initial worsening of some soil parameters at the start of the transition to CA, the results of this study showed a progressive positive evolution under reduced tillage management during the first three years after conversion from conventional agriculture to CA. In particular, the introduction of sod seeding practices resulted in the most effective strategy to gain CA advantages since the first year after conversion. Neither physical nor biochemical indicators seemed negatively affected during the transition phase. The NT treatment exhibited the best results, relative to the other treatments tested, for improved environmental sustainability. However, if yield alone was considered, then NT seemed particularly sensitive to crop failure under adverse conditions. In fact, sod seeding (essentially NT) under very poor climatic conditions, suggested a higher risk of crop failure.

The interaction between tillage intensity and soil covering was limited; cover crop effects were always less evident relative to tillage effects. Moreover, the expected benefits of TR were detectable only under controlled conditions (see Chapter 4). Possible explanations of these results could be: i) CC soil carbon inputs benefits are not evident in the short term; ii) expected TR effects, mainly related to biopore creation, which requires a complete root degradation, may not be evident prior to subsequent crop sowing or could fade after main crop harvest. Therefore, the expected benefits of tillage radish CC combination with reduced tillage systems were not observed. Furthermore, the short-term improved soil conditions arise mainly from the natural evolution of undisturbed soil and, possibly, to the increased activity of soil fauna.

The introduction of a CC into a crop rotation requires careful evaluation, not only of timing, but also of species, agronomic techniques and CC environmental effects. To minimize the costs of CC management, September was the best option for Brassicaceae species. Poaceae species, like WW, can be sown later and still provide good coverage. In the case of TR CC, this recommendation indicates that the previous cash crop must be harvested sufficiently early, as a delayed seeding may limit CC development, and lead to reduced CC benefits and induced frost resistance. For spring vegetative restarts, necessary chemical or mechanical devitalization will increase management costs, but may be preferable to the risks associated with a sowing delay of the subsequent cash crop or an uncontrolled weed germination.

Soil under CA, especially in the first years of transition, exhibited high micro-scale variability. Scale can affect the measurement reliability of PR and (to a lesser extent) BD, since they were investigated in a relatively small area. On the other side, methods such as the double ring infiltrometer seemed less affected by this variability: in fact, it is performed on a wider area, resulting a better indicator of the physical evolution of the soil under CA. The usefulness of BD and PR thresholds to evaluate the potential root growth can then be a useful guide for homogeneous profiles, such as a tilled soil, but become inadequate in a more differentiated system as those found in NT.

To conclude, no tillage resulted the pivotal strategy to implement the CA and obtain the expected benefits since the first years. The main risk related with the introduction of the NT practices is the higher sensitivity to adverse biotic and meteorological conditions which must be evaluated, especially in the perspective of the climate change. Site specific studies could contribute to determine the best strategies to contrast these adversities, ensuring the CA economic sustainability.

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