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Title:

Short-term dynamic responses of soil properties and soil fauna under contrasting tillage systems

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Highlights

- 15
 - Reduction of tillage led to an improvement of the soil physical structure.
 - The sampling time since tillage can strongly affect the soil food webs.
 - Repeated tillage keeps soil fauna at an early stage of ecological succession.
 - In the short term, Collembola are more resilient to soil disturbance than earthworms.

Abstract

20 Achieving sustainability is a worldwide current concern in agriculture that brings important challenges but also opportunities for rethinking agroecosystems. While many studies focused on tillage effects upon soil organisms, few of them have considered this into a temporal framework. In a study conducted in actual field conditions, we evaluated at short-term (7 months) the dynamic of several abiotic and biotic soil parameters under contrasting tillage systems. We hypothesized that (1) the reduction and especially the stopping of the soil mechanical perturbation lead overall to an improvement of the soil physical structure and the food resources available for soil fauna. (2) According to their specific ecological requirements, Collembola and Earthworms will show different dynamic of responses following alleviation of soil mechanical perturbation. (3) For a single group (either 25 Collembola or earthworms), differences between tillage systems are dependent on the sampling period. We used a field set-up held on the INRA experimental station of Estrées-Mons (North of France). Three tillage systems were investigated: conventional tillage (with the soil inverted up to 25 cm depth) reduced tillage (limited to the first 7- 8 cm depth) and no-tillage. Each treatment was replicated 4 times randomly (3 x 4 =12 plots in total). Collembola 30 and earthworms were sampled at each plot using standard methods. During the course of the experiment, two tillage operations were performed one in mid-March and one in mid-May. Samplings of soil fauna were performed on a regular basis according to tillage operation: 1 day before, and respectively 1; 7; 30; 49; 56; 104; 210 days after. Abiotic parameters (bulk density, SOC stock, Mean Weight Diameter (MWD) and aggregates size) and microflora 35 (microbial and fungal biomass and their activity) were also monitored and used as explaining factors. Our findings showed that slightly reducing the intensity of soil mechanical perturbation, did not favour soil fauna. But, when stopping tillage, population sizes of earthworm and Collembola increased. This is related to the improvement of the distribution 40

of, and accessibility to, organic matter as a basic food source (SOC stock, microbial and
45 fungal biomass and their potential C mineralisation activity), the physical structure of the soil
and the distribution of pore sizes as a space life (bulk density, MWD and aggregates size).
Our kinetic study has shown that the different groups of soil fauna respond differently to
mechanical soil disturbance. Overall, conservation tillage can foster one of the numerous
services provided by the soil compartment, namely the soil biodiversity and therefore improve
50 soil quality and health.

1. Introduction

Human activities, among them, land use and soil management are major drivers exerting pressures on soils and ecosystem services they provide (Smith et al., 2016). In agrosystems, although conventional tillage (CT) is an effective practice in repressing annual weeds and facilitating precise seeding (Ehlers and Claupein, 1994), its application is often accompanied by the destruction of the habitat of edaphic organisms, preventing agro-ecosystem self-organisation (Altieri, 1991; Brussaard et al., 2007; Holland, 2004; Six et al., 2000; Tsiafouli et al., 2015). Thus, a main goal in soil research is to rethink agroecosystems to achieve sustainable and profitable methods that may improve livelihoods of farmers and the best possible utilization of naturally occurring soil processes (DeLong et al., 2015; Kassam et al., 2010; Tittonell, 2014; Wezel et al., 2014). To do so, it has been suggested that agrosystems should imitate natural ecosystems (Jackson, 1980; Scherr and McNeely, 2007), through the application of three fundamental principles of Conservation Agriculture (CA): "minimal mechanical soil disturbance, permanent soil cover and crop rotations" (FAO, 2015; García-Torres et al., 2013; Hobbs, 2007; Knowler and Bradshaw, 2007). Indeed, the main goal of CA ranges from reducing the ploughing depth (reduced or superficial tillage, RT) while retaining crop residues on or near the soil surface (Brown and Tworkoski, 2004; Lal, 2013), to direct seeding without any soil tillage (no-till, NT), whose the only soil disturbance being caused by seeding and during harvest (Kassam et al., 2009).

Conversion from CT to RT and/or NT was considered to be one of the potentially efficient strategies to achieve more sustainable agricultural practices. For example, reducing tillage frequency and intensity may lead to reduced carbon emissions due to savings in machinery and energy use (Bank, 2007; Lahmar, 2010). It may also allowed a higher C sequestration rate

(Lal, 2004), which could protect soils from erosion and compaction (Holland, 2004; Lugato et al., 2014). Reducing tillage was also shown to improve soil porosity, temperature and humidity while causing minimum damage to edaphic organisms (Derpsch et al., 2010; Hobbs, 2007; Mazvimavi et al., 2008; Stewart, 2007), thereby favouring biological nitrogen fixation (Huggins and Reganold, 2008), raising and/or stabilizing yields and also lowering production costs (Kassam et al., 2010; Lahmar, 2010).

Soil biota and the processes they drive have large direct and indirect effects on crop growth, soil- and residue-borne pests, nutrient cycling and water transfer (Lavelle et al., 2006). The conservation of belowground diversity has become a key component of a strategy towards agricultural sustainability (Bender et al., 2016; Byrnes et al., 2014; Nielsen et al., 2011). To develop cropping practices that ensure an optimal use and protection of soil biodiversity, the main challenge is to predict the impacts of tillage systems on organisms and to understand the links between ecosystem processes and services they provide (Brussaard et al., 2007; Creamer et al., 2016; Temme and Verburg, 2011). The principal differences between tillage systems involve the depth of tillage (as just described), frequency of the tillage operations (per annum or per seasons) and manipulation of weed levels (Wardle, 1995). Accordingly, properties and habitat conditions in soil, which partly drive biodiversity belowground, differ as well. Abundance, diversity and activity of soil biota, in this context, are hypothesized to be mainly affected by distribution of organic matter as basic food source, soil structure and pore size distribution as available living and moving space, air and water movement as environmental milieu, and indirectly through modified interactions within the soil food web (de Vries et al., 2013; Wardle, 1995). The adoption of less intensive soil cultivation practices is expected to favour soil biota and their contributions to ecosystem functioning (Briones and Schmidt, 2017; van Capelle et al., 2012). However, more sustainable managed systems do not always result in an increase in soil fauna abundance and

diversity (Berner et al., 2008; Chan, 2001; van Capelle et al., 2012). This conflicting picture might be the result of failing to examine the effects of tillage intensity under a broader scheme including the levels of tillage, the number (secondary tillage/surface tillage after mouldboard tillage) and frequency of the tillage operations (per annum or per seasons) during a growing
105 season (Capowiez et al., 2009; Ivask et al., 2007), the crop phenology (Barbercheck et al., 2009), as well as variations of climatic factors (e.g. temperature, rainfall) during a vegetative season (Bertrand et al., 2015; Chan, 2001). Furthermore, several studies on the impact of tillage on soil processes have shown that different parameters such as soil fauna, dynamic of soil organic matter (SOM) or soil structure responded differently to the tillage system from
110 the time of till to crop growth (Bedano et al., 2006; Boizard et al., 2017; Petersen, 2002). All these plead in favour of improving our knowledge of soil tillage impact on soil biodiversity and abiotic parameters during a growing season and its consequence on associated ecological functions.

In the present study, we monitored earthworms and Collembola assemblages over a growing
115 season in response to different practices varying in tillage intensity (CT, RT and NT) on a field that has been under CT for at least 20 years. Earthworms and Collembola, with different life-cycles and sizes, are the most abundant groups of macro and mesofauna, respectively, in agricultural systems (Chauvat et al., 2014; van Capelle et al., 2012). Both are of rather large size, living in air-filled pores, require a network of pathways for movement, and thus more
120 sensitive to habitat disruption of pore networks. They are obviously important for the fertility of agricultural soils through mainly soil structuring (Shipitalo and Protz, 1989), the transformation of organic matter and the recycling of nutrients by the regulation of microbial communities (Filser, 2002; Kaneda and Kaneko, 2008; Petersen, 2000). In tilled agrosystems, there tend to be fewer earthworms. These large animals are often physically damaged by the
125 plough-line or during soil inversion they may be brought to the surface and being consumed

by different birds or other predators. Additional meta-analyses confirmed that epigeic and, more importantly, the bigger-sized anecic earthworms were the most sensitive ecological groups to conventional tillage (Hendrix et al., 1986). Endogeic earthworms could be favoured if tillage operations alleviate soil compaction and incorporate crop residues serving as food resources (Boström, 1995; Whalen and Sampedro, 2010). The smaller-sized Collembola are less prone to be directly physically damaged by tillage implements (Wardle, 1995). Although tillage effect on soil Collembola has not been studied extensively, they tend to be negatively impacted (Whalen and Sampedro, 2010). Finally, response of earthworms and Collembola in agrosystems might differ according to chemical weed control, as the earthworm epidermis allows toxins to diffuse through, whereas some collembolans have a hydrophobic chitin cuticle.

Our main objective was to study the response dynamic of these two groups of soil organisms and their links to soil physico-chemical and microbial properties as a food resource when shifting from conventional tillage towards less intensive management practices (reduced-tillage and no-tillage). We hypothesized that (1) the reduction and especially the stopping of the soil mechanical perturbation lead overall to an improvement of the soil physical structure and the food resources available for soil fauna. (2) According to their specific ecological requirements, Collembola and Earthworms will show different dynamic of responses following alleviation of soil mechanical perturbation. (3) For a single group (either Collembola or earthworms), differences between tillage systems are dependent on the sampling period (i.e time elapsed since tillage operations).

150 **2. Materials and Methods**

 2.1. Site description

We used a field set-up held on the INRA experimental station of Estrées-Mons (North of France). Soil was a “LUVISOL typique” according to the “Référentiel pédologique 2008” (Baize and Girard, 2009), equivalent to LUVISOL in the World Reference Base (FAO, 2006).

155 Initial soil physico-chemical parameters measured on the depth of 0-20 cm indicated: clay = $187 \pm 31 \text{ g.kg}^{-1}$, silt = $744 \pm 34 \text{ g.kg}^{-1}$, sand = $69 \pm 21 \text{ g.kg}^{-1}$, pH in water = 8.20 ± 0.10 , organic C = $10.40 \pm 0.80 \text{ g.kg}^{-1}$, and total N = $1.04 \pm 0.05 \text{ g.kg}^{-1}$. Climate is temperate with an annual average rainfall of 678 mm and an average annual temperature of 10.8 °C.

The experimental design was established on an area of 12 by 100 m, which was under
160 conventional tillage (mouldboard ploughing, depth = 25cm) with crop rotation for several decades before the beginning of the experiment. In spring 2014, instead of a single conventional tillage on this plot, we introduced three levels of soil preparation, which are common practices: conventional tillage (CT) with the soil inverted up to 25 cm depth by
165 mouldboard ploughing, reduced tillage (RT) - i.e. shallow tillage - limited to the first 8 cm of soil depth using disk ploughing and no-tillage (NT) without any mechanical disturbance. Each system (i.e level) was replicated 4 times in blocks (12 sub-plots in total, each sub-plot is 9 m long and 6 m wide; Fig.1). The sub-plots were separated by 6 m wide buffer strips (Fig.1).
During the experiment, two tillage operations were performed on the sub-plots CT and RT, one at the end of March after the winter and one in mid-May corresponding to the seedbed
170 (*Zea mays, L*) establishment. The seedbed establishment corresponds to a reduced tillage. NT was sown manually without any soil mechanical preparation. Glyphosate was applied to all sub-plots (NT including) just prior to sowing (3 days before the seed beds) to remove weeds.

Standard sensors were used to continuously monitor rainfall and soil temperature over the study period (Supplementary material - Fig. S1).

175 2.2. Soil physico-chemical properties

Soil samplings were performed on a regular basis according to the first tillage operation: 1 day before, and respectively 1, 7, 30, 49, 56, 104, 210 days after. Highlight between D0 and D1, we noticed frozen on the soil surface. Soil moisture (SM) was measured on cool soil by differentiating between wet soil weight and dry soil weight after drying at 105
180 °C for 24 hours. Soil pH_{water} was measured with dry soil (sieved at 2 mm) diluted with demineralized water (NF ISO 10390). Bulk density (BD) was determined by taking two undisturbed soil cores from each sub-plot from 0- to 5-cm depth. We took the cores by using a metal cylinder of 100 cm³. The cylinders were sealed and transported to the laboratory. Thereafter, the collected soil samples were weighed, and then dried at 105°C until constant
185 weight during a period of approximately 24 h. BD is equal to the dry mass of soil contained in the metal cylinder divided by the volume of the cylinder (Guo and Gifford, 2002). BD is expressed in g cm⁻³, which is numerically equal to BD expressed in SI unit Mg m⁻³.

For all analyses excepted bulk density, sampled soils were air-dried until constant weight then sieved at 2 mm. An aliquot of each sieved soils was grinded and powdered for
190 Total Carbon analysis measured by gas chromatography with Thermo scientific Flash 2000 CHN S / O analyzer. We then measured the Soil Organic Carbon (SOC) by making the correction of the TC by the mineral carbon (CaCO₃). Subsequently, we were able to calculate SOC stocks according to the following formula (Mann, 1986): SOC stock (kgC.m⁻²) = SOC (kgC.kg of soil) x BD (kg of soil.m⁻³) x D (m); where D is soil sampling depth. The SOC
195 stock was measured on the depth of 0- to 0.05m.

For aggregates size distribution, we have collected an intact soil cores of 8 x 8 x 10 cm in each sub-plot. The intact soil cores were transported and stored in plastic boxes closed and handled carefully to avoid destroying the soil structure. In the laboratory, the samples were passed through an 8 mm mesh-sieve and dried in ambient air for one week. Subsequently, we used a dry sieving method according to Robertson et al. (1999), that consists in taking 50 g of dry soil pre-sieved to 8 mm and placing it in an automatic sieving machine RETSCH AS 200 digit comprising various sizes of sieves (5 mm, 2 mm, 0.250 mm, and 0.05 mm). For each sample, the machine was calibrated in continuous vibration with amplitude of 2 mm and duration of 5 min. We then recovered five aggregates fractions of different sizes: > 5 mm, 5 to 2 mm, 2 to 0.25 mm, 0.25 to 0.05 mm, and clays and silt less than 0.05 mm. All fractions were dried in an oven at 60 °C for 24 h and their weights were noted thereafter. At the same time, we measured the residual moisture by taking 10 g of the soil sieved at 8mm and dried it in an oven at 105 °C for 24 hours. We calculated the size of the Macro-Aggregates (MAG) by summing the aggregates > 0.250 mm. Finally, aggregates' Mean Weight Diameter (MWD) was calculated from the size distribution (Van Bavel, 1950). $MWD = \sum (\varnothing \text{ mean between 2 sieves} \times [\% \text{ weight of particles retained on sieve}]) / 100$.

2.3. Microbial, fungal biomasses and potential C mineralization

At each of the 12 sampling points for each date, two soil cores were taken to a depth of 10 cm and then pooled to obtain one composite sample per plot. All composite soil samples were placed in plastic containers, stored in cool boxes at 4 °C and transported to the laboratory for microbial analyses. In the laboratory, composite soil samples were sieved at 2mm.

The microbial biomass carbon (MBC) was estimated by means of the fumigation-extraction method (Jenkinson and Powlson, 1976). Basically, before and after fumigation, 20

220 g of soil were shaken for 1 h in a solution of K_2SO_4 at 0.05M then filtered at 0.45 μg and analysed for dissolved organic C on Shimatzu-TOC-5050A.

Ergosterol concentration, used as a proxy for fungal biomass, was determined according to the method of Gong et al. (2001). Briefly, 4 g of moist soil was added to a vial (20 ml) containing 4 g of acid washed glass beads and 6 ml of methanol. The vial was stirred
225 on an orbital shaker for 1h at 320 rpm. After shaking, the soil mixture was allowed to sediment for 15 min. An aliquot of 1.5 ml of supernatant was centrifuged for 10 min at 11000 rpm at 5°C. The supernatant was filtered (0.2 μm) and injected for the HPLC analysis (High-Performance Liquid Chromatography, SPD-10A SHIMADZU LC-6A, Japan), using UV detection at 282 nm.

230 The potential C mineralization rate was measured as CO_2 evolution from 50 g aliquots of sieved fresh material incubated under conditions of 28°C and 80% of field capacity for a 7 days period in hermetic pots. CO_2 released was captured by NaOH (0.2 M) and measured by conductivity meter (Thermo Scientific, Orion 4 Star).

235 2.4. Soil fauna samplings, extraction and identification

As for soil physico-chemical properties, samplings were performed on a regular basis according to the first tillage operation: 1 day before, and respectively 1, 7, 30, 49, 56, 104, 210 days after (Figure 1).

2.4.1. Collembola

240 For each of the twelve (12) plots and at each sampling date, two soil samples were collected by means of soil corers (diameter: 5 cm). A single core included the organic or organo-mineral layer and the top 10 cm of the mineral horizon. In the laboratory, Collembola were

extracted during 10 days using a Berlese-Tullgren device that creates a temperature gradient over soil cores, crumbled into a plastic 2mm sieve, suspended over a collecting vessel
245 containing 70% alcohol. Individuals (Collembola) collected in alcohol were counted and identified following keys of determination. However, several individuals were only identified to the genus level when species identification was not possible.

2.4.2. Earthworms

Conventional methods of sprinkler extraction of irritating substance could not be used as part
250 of a temporal monitoring of experimental plots at the risk of making them unfavorable to the further reception of fauna. Therefore, one 25 cm × 25 cm × 25 cm monolith was hand sorted for earthworms in each plot and for each sampling date. Specimens were fixed in pure alcohol and categorized as juveniles (ac clitellates) and adults (clitellates). Adults were identified to species level according to Sims and Gerard (1999). Juveniles were therefore not taken into
255 account for the estimation of species number but were when calculating total earthworm densities expressed as number of individuals per m².

2.5. Statistical analysis

Firstly, statistical analyses focused on the data collected for each date separately (D0 to D210), in order to compare the experimental treatments over time (treatment effect).
260 Secondly, we performed analyses to quantify changes between sampling dates for a single treatment (sampling day effect). To investigate the effect of tillage practices on soil fauna, soil abiotic and microbial parameters, we used Linear Mixed-Effects Models (LMM) with the descriptive variables of Collembola and earthworms (total density, total species richness, diversity index), or environmental parameters as response and the factor “treatments” (CT, RT, and NT) as fixed categorical predictor, the factor “experimental blocks” (B1, B2, B3, and
265 B4) as random categorical predictor. Significant differences between means were tested at a

5% rejection probability level using the Tukey HSD test. Normality and variance homogeneity were tested prior to statistical analyses.

We finally performed a Redundancy Analysis (RDA) to visualize relationships between soil fauna community variables (total density and species richness of Collembola and earthworms) as a response matrix (Y) and abiotic and microbial parameters as an explanatory matrix (X). RDA (Legendre and Legendre, 2012; Rao, 1964) is an extension of multiple regressions that allows one to explain (in the same sense as in regression analysis) the variation of a multivariate response data table using explanatory variables. A linear function of time allowed us to model the variation in soil fauna communities among sampling dates in a circle of correlation. We have thus analysed separately the three tillage treatments, to see the specific effect of each of them. For this, we used *rdaTest* function (Legendre and Durand, 2010). This allows a canonical analysis of simple and partial redundancy with permutation tests and the production of triple projection graphs. The analysis (*rdaTest*) is suitable for the design of repeated measures over time. The *rdaTest* were computed on log-transformed data, $y' = \log(y+1)$.

The calculations were performed in the R language (Team, n.d.) with the libraries *ade4* (Dray and Dufour, 2007), *lme4* (Bates et al., 2014), *MuMIn* (Bartoń, 2013), *multcomp* (Hothorn et al., 2008), *car* (Fox and Weisberg, 2010) and *vegan* (Oksanen et al., 2013). Redundancy analyses were computed with the *rdaTest* package available on <http://adn.biol.umontreal.ca/~numeralecology/Rcode>.

3. Results

3.1. Soil physico-chemical properties

3.1.1. pH water

290 Soil pH water values neither differed over time nor between treatments (cf. Supplementary material- Table S1). They ranged between 7.9 and 8.2.

3.1.2. Soil moisture (SM)

Soil moisture (SM) showed significant temporal variations in all tillage treatments (sampling day effect, Table 1, Fig.2). The maximum and minimum values of soil moisture were recorded at D7 and D56 for CT and RT, and at D7 and D30 for NT treatment.

SM differed significantly between treatments at two sampling dates: D30 and D56 with RT treatment showing significantly higher values than the two other treatments (treatment effect, Table 1, Fig.2).

3.1.3. Bulk density

300 Soil bulk density did not differ with time in NT treatment, but did significantly in CT and RT treatments (sampling day effect, Table 1, Fig.3). The maximum and minimum values of CT were recorded at D1 and D49; and at D0 and D56 for RT treatment.

This parameter differed significantly between treatments at all sampling dates except for D0 and D210. NT had the highest values except for D1 where the maximum value was recorded in CT (treatment effect, Table 1, Fig.3).

3.1.4. SOC stocks

As bulk density, SOC stocks showed significant temporal variations in CT and RT treatments, but not in NT (sampling day effect, Table 1, Fig.4). For both CT and RT treatments, maximum and minimum values were recorded at D0 and D7. SOC stocks differed significantly between treatments at all sampling dates except at D0, D1, and D210. NT treatment had significantly higher values than the two other treatments (treatment effect, Table 1, Fig.4).

3.1.5. Mean weight diameter (MWD)

MWD differed significantly between sampling dates in both CT and RT treatments, but not in
315 NT (sampling day effect, Table 2). For CT, maximum and minimum values were found
before tillage (D0) and after germination (D104), respectively. Accordingly, MWD in RT did
not change significantly after tillage, differences were observed after the seedbed (D56) and
germination (D104) where we recorded the lowest values, 2.54 and 2.42 mm, respectively. As
for “treatment effect”, this parameter differed statistically between treatments only at D30,
320 D56, and D104, with higher values in NT than in CT and RT except at D56, where RT
exhibited intermediate values (Table 2).

3.1.6. Microbial biomass carbon (MBC)

MBC varied widely over time in all treatments (sampling day effect, Table 3). For CT, higher
values of MBC were observed at D0 and D210 compared to the value recorded at D56. For
325 both RT and NT treatments, highest MBC was recorded at D1 and lowest at D7.

MBC differed significantly between treatments at several sampling dates at D1, D7, D30, and
D49 (treatment effect, Table 3). On average, highest values were generally found in RT and
NT treatments except at D7 where CT had a higher value. No difference was found before
tillage (D0) and after seedbed until harvest (i.e D56, D104, and D210).

330 3.1.7. Fungal biomass

As MBC, fungal biomass varied significantly over time in all treatments (sampling day effect,
Table 3). For CT and RT, minimum values were found 7 days after tillage (at D7) and
maximum values were found after seed bed (at D56; D104) in CT and at D49; D56; D104 in
RT. For NT, maximum and minimum values were recorded at D49 and D30, respectively.

335 Accordingly, fungal biomass was influenced by experimental treatments at D1, D30, D49,
and D104, with always higher values measured in RT compared to CT and NT.

3.1.8. Potential C mineralization

Potential C mineralization showed significant temporal variations in all treatments (sampling
day effect, Table 4). In CT, maximum values were found at D1 and minimum values at D0
340 and D7, the other days exhibiting intermediate values. In RT, highest value was found at
D104 and lowest values at D0 and D210. In NT, maximum value was recorded at D49 and
minimum values were found at D0 and D210. The factor "treatment" significantly impacted
the Potential C mineralization at D7, D30, D49, and D104, with significantly lower values in
CT compared to the two other tillage systems (RT and NT).

345 3.2. Soil fauna

3.2.1. Collembola

Total density, total species richness and Shannon index (biodiversity indices) of Collembola
varied significantly over time in each of the three tillage treatments (sampling day effect,
Table 5). In CT, these parameters have higher values at D0, D30, and D210 and lower values
350 at D56 (seed bed) and D104 (germination). In RT, higher values of all parameters were found
at D0, D30, D49 than other sampling days; D210 exhibiting intermediate values. For NT
treatment, lowest values were found at D7 and D104 (total density) and only at D104 (total
species richness and Shannon index; Fig. 5, 6, and 7).

All parameters were impacted by the factor "treatment". Total density significantly differed
355 between the treatments on three occasions (treatment effect, Table 5). At D49, on average, 4-
fold more individuals were collected in NT and RT than in CT (Fig.5). After seedbed (D56),
the total density was about 6-fold higher in NT compared to CT and RT (Fig.5). Finally, at

D104, total density ranged from *ca* 510 to 3312 individuals m⁻² (in CT and NT, respectively), RT showed intermediate values (Fig.5).

360 Total species richness and Shannon index displayed the same pattern, but only differed between treatments at D56 and D104 (treatment effect, Table 5). At D56, just after seedbed, the Collembola species richness and Shannon index were significantly higher in NT compared to CT and RT. At D104, a different pattern was observed with significantly higher species richness and Shannon index in RT and NT than in CT (cf. Supplementary material -
365 Fig. S2 and Fig. S3).

3.2.2. Earthworms

The factor “time” significantly impacted all diversity parameters (total density, total species richness and Shannon index) of Earthworms in each of the three treatments (sampling day effect, Table 5). For CT and RT, significantly higher values of total density were recorded at
370 D0 compared to all the other sampling dates. Then, the values of all parameters decreased progressively over time, with lower values observed at D56 in CT and at D210 in RT (Fig. 6). Total species richness and Shannon index displayed the same pattern, being significantly lower at D49 and D56 in CT and D56 and D210 in RT (cf. Supplementary material - Fig. S4 and Fig. S5). For NT, total density was higher at D0 and D7, lower values recorded from D56
375 to D210 (Fig. 6). Total species richness and Shannon index in NT showed higher values at D0, D1, and D7 compared to values observed at D210 (Fig. S4 and Fig. S5).

The total density of earthworms differed significantly between treatments at all sampling dates after tillage (from D1 to D210, treatment effect, Table 5). During the study, the highest densities of earthworms were recorded in NT treatment compared to CT and RT (Fig. 6).
380 Also, we noted that the density was significantly lower in RT than in CT on several dates such as D7 (88 vs. 132 individuals m⁻²), D30 (44 vs. 84 individuals m⁻²), D104 (28 vs. 48

individuals m⁻²), and D210 (20 vs. 44 individuals m⁻²). The total species richness and Shannon index of earthworms were also significantly influenced by the factor "treatment" from D1 till D56 (Table 5), with higher values in NT compared to both CT and RT (Fig. S4 and Fig .S5).

3.3. Relationships between soil fauna, soil physico-chemical parameters and microflora

3.3.1. Conventional tillage (CT)

In CT system, the proportion of the total variance in the RDA, which is explained by all environmental parameters, is 37.2%. The first axis accounted for 91.1% of variance and the second only for a further 6.67% (Table 6). Basically, the first axis shows temporal variations after tillage operations (tillage and seedbed, see Fig.7). D0; D30 and D210 correlating to high values of collembolan assemblages. Both total density and species richness of Collembola were related to higher values of SOC stock, mean weight diameter (MWD) and soil moisture (SM). In opposite, sampling dates D1 and D7 were associated to high values of microbial biomass carbon (MBC), total density and species richness of earthworm. These latter were related to higher values of bulk density (BD).

3.3.2. Reduced tillage (RT)

For RT treatment, the proportion of the total variance in the RDA, which is explained by all environmental parameters, is 20.3%. The first axis accounted for 53.1% of variance and the second only for a further 21.8% (Table 6). The first axis shows effect of mechanical perturbation (Fig.8). The first axis opposes D0 and D56, with high values of macro-aggregates (MAG) and potential C mineralization at D56. On the second axis, firstly, D30, D49, D104, and D210 were associated to high values of collembolan assemblages, microbial biomass carbon (MBC) and fungal biomass (FB). These latter were related to high values of

soil moisture (SM), SOC stock and bulk density (BD). On the other hand, D1 and D7 were correlated to high values of total density and species richness of earthworm, which were related to high values of mean weight diameter (MWD).

3.3.3. No tillage (NT)

410 In NT system, the proportion of the total variance in the RDA, which is explained by all environmental parameters, is only of 2.71% hindering robust conclusions about it. The first axis accounted for 20.0% of variance and the second only for a further 6.01% (Table 6). Both axes showed temporal variations along the study (Fig.9).

4. Discussion

415 We followed during a full vegetative season the effects of 3 different tillage systems commonly used in Western Europe. Since all the studied sub-plots were previously managed under conventional tillage (CT) for at least 20 years, this offered us the opportunity to follow during a growing season if shifting from CT towards less intensive management practices (by minimizing mechanical disturbances) may produce changes in soil fauna assemblages
420 impacting ecological processes. Furthermore, we also aimed at depicting how the sampling time since tillage may modulate the responses of soil fauna to different tillage systems.

4.1. Soil physico-chemical parameters and food resources

Bulk density, SOC stock, MWD, pH, potential C mineralization, microbial biomass carbon (MBC) and fungal biomass (FB) values obtained in our study were within the range of
425 values observed in similar studies within Europe (Lampurlanés and Cantero-Martinez, 2003; Van Groenigen et al., 2010).

Slightly reducing the intensity of soil mechanical perturbation (RT) did not impact abiotic factors. However, when stopping tillage and soil preparation for seedbed, then bulk

density, stock C, MWD, increased (NT vs. CT). This partially confirms our first hypothesis
430 and fits with the common assumption that mechanical perturbation like tillage is the major
cause of physical habitat alteration leading to soil degradation, i.e. organic matter depletion
and reduction of soil fertility (Montgomery, 2007; Reynolds et al., 2007; Six et al., 2000). The
decrease in soil bulk density observed at D1 (1 day after tillage) in NT vs CT was due to the
inversion of the soil by CT, which may alter this condition in short term.

435 Our results showed that between D0 and D1, potential C mineralization increased in
RT and CT and no effect was detected for NT. This increase did not last long, since 7 days
after tillage we observed a subsequent decrease in these systems especially in CT. This result
is in perfect agreement with those found by Calderón et al. (Calderon et al., 2001), showing a
CO₂ increase in soil lasting only 4 days after tillage. Indeed, tillage is known to induce short-
440 lived increases in CO₂ efflux from soil. Several authors noticed that immediately after tillage,
the CO₂-rich soil atmosphere is flushed with air of relatively low CO₂ content. This causes a
reduction in the CO₂ partial pressure in the soil air, which is followed by the degassing of
dissolved CO₂ from the soil solution (Ellert and Janzen, 1999; Reicosky et al., 1997; Reicosky
and Lindstrom, 1995; Rochette and Angers, 1999). When we look at our kinetic study,
445 reducing and stopping tillage has led to an increase in potential C mineralization (D30, D49,
D104). We hypothesize that factors such as the intensity of soil disruption, the amount of
initial microbial biomass, and the quantity of previously protected C sources in soil
aggregates may determine the impact of tillage on microbial activity.

On the other hand, our results showed that microbial communities are reactive to
450 tillage intensity. The reduction of mechanical disturbance has led to an increase of fungal and
microbial biomass (FB and MBC) from the first day after tillage, followed by a decrease 7
days later. This is in agreement with the findings of several studies (Salinas-García et al.,
2002; Van Groenigen et al., 2010; Zhang et al., 2015), but not with the results of Calderón et

al. (Calderon et al., 2001), which showed a lack of reduced tillage effect on microbial biomass
455 in the short term. Due to its dynamic characteristic, microbial biomass quickly responds to
changes in soil management, because it has the advantage of serving as signals of soil
structure improvement (Powlson et al., 1987). Shallow non-inversion tillage is carried out
using a single pass of a tined stubble cultivator at a depth of 7–10 cm, this may cause an
increase in fungal hyphae on the soil surface; thus, it can be suggested that the reduction of
460 tillage may expose previously protected organic matter to microbial communities, enhancing
their growth (Das et al., 2018; Kabiri et al., 2016; Zuber and Villamil, 2016).

Finally, at D56, all microbial parameters (MBC, FB and potential C mineralization)
did not differ between the three treatments. This could be partly explained by the application
of glyphosate in all the treatments just before D56 to manage weeds. Glyphosate degradation
465 could be co-metabolically performed by the general activity of soil microorganisms (Araújo et
al., 2003; Haney et al., 2000; Ratcliff et al., 2006; Stratton and Stewart, 1992). Several
authors have studied the effects of glyphosate on soil microorganisms, with inconsistent
results. While, Gomez et al. (Gomez et al., 2009) observed a decrease of microbial biomass
carbon, Haney et al. (Haney et al., 2000) did not on neither the microbial biomass carbon nor
470 nitrogen contents, and yet, Wardle and Parkinson (Wardle and Parkinson, 1990) found a
slight increase in these parameters in the first days after application of this herbicide. We also
cannot exclude the potential impact of climatic conditions levelling off the differences
between the treatments. Unfortunately, our experimental field design did not allow us to
control all variables to clearly identify the mechanisms responsible for the changes found at
475 this sampling period.

However, it is interesting to note that at the last sampling date (just before maize
harvest, D210), all these parameters revert to the same level as NT. This could be related to
vegetative growth mainly through root development. For example, Bardgett et al., (2014)

showed that root density and depth of rooting have potentially important implications for soil
480 formation and stability. These, notably because of their significant contribution to the
improvement of apparent density by the reduction of soil porosity, as well as to the
sequestration of carbon in the soil (De Deyn et al., 2008; Gyssels et al., 2005). Furthermore,
many studies have shown how soil microbial communities may be fuelled within the
rhizosphere. Thereby, we suggest, that maize growth during our experiment, and especially
485 through its rooting system, may have been a stronger driver than tillage practices on soil
structure and ecological processes.

4.2. Soil fauna

The average number (density and species richness) of earthworms and Collembola
490 collected in all plots are comparable with former published data from similar systems in
France (Cortet et al., 2002; Henneron et al., 2014; Ponge et al., 2013) and other parts of
Europe (Briones and Schmidt, 2017; Petersen, 2002; van Capelle et al., 2012).

Overall, results of our study demonstrate that assemblages of earthworms and
Collembola are reactive to tillage intensity. The lower the soil tillage intensity, the higher the
495 Collembola or earthworm density. This effect was consistent across all parameters analysed
(density, species richness and Shannon index), which is in accordance with a number of
previous studies (Briones and Schmidt, 2017; Petersen, 2002; Sousa et al., 2006; van Capelle
et al., 2012; Wardle, 1995). This agrees well with a general view that soil biota is usually
stimulated by new conservation tillage practices. Nakamoto et al. (Nakamoto et al., 2006)
500 found the same results about the numbers of nematodes, but for example Didden et al.
(Didden et al., 1994) indicated that enchytraeids were unaffected by tillage. However, our
kinetic study allowed us to show that Collembola assemblages (density, species richness and

Shannon index) differed only after 49 days (D49) between the tillage treatments (for total density) and the second tillage operation (i.e seedbed, the total densities, richness and Shannon index), unlike earthworms with significant differences observed already after the first tillage operation (from D1). Our study, as we postulated (second hypothesis) revealed that Collembola and Earthworms showed different dynamic of responses following alleviation of soil mechanical perturbation. Generally, larger organisms are more sensitive to tillage practices than smaller ones (Wardle, 1995). For example, Briones and Schmidt (Briones and Schmidt, 2017) stated that when a soil is ploughed, earthworms are either injured and killed directly or exposed to adverse environmental conditions and predators (Giller et al., 1997). Their biogenic structures (pores and burrows) are destroyed and their food sources (litter) buried (Chan, 2001). We suggest that Collembola may be more resistant than the earthworms to ploughing and that the repetition of the tillage (i.e seedbed) causes them to decrease afterwards. The fact that the total densities of Collembola differed significantly between the three soil tillage systems at D49, with higher values in RT and NT compared to CT, is directly related to the effect of tillage as described for earthworms above, but also indirectly through habitat modification, as can be seen with increasing soil moisture values at D49 (cf. Fig. 2 & Fig. S1). In one way or another, the effect of tillage on Collembola assemblages remains delayed compared to earthworms. This supports our third hypothesis suggesting that the difference between tillage systems is related to the sampling period. In the literature, studies have often compared the effect of these three tillage systems on Collembola assemblages (Cortet et al., 2002; Dittmer and Schrader, 2000; Petersen, 2002; Wardle, 1995; Winter et al., 1990). They stressed that the latter are promoted by the NT system compared to other tillage systems. However, they often failed to provide information at what time of the season the differences may appear. Nevertheless, Barbercheck et al., (2009), investigating the potential of soil micro-arthropods as bioindicators of soil conditions in disturbed sites,

addressed this seasonal effect with tillage as one of the types of disturbance and they concluded that no single seasonal measure reflected disturbance.

530 It is surprising that the total earthworm density was significantly lower in RT than in CT at several sampling dates (D7, D30, D104 and D210). This is in contradiction with the results found by van Capelle et al., (2012) in a meta-analysis, but is in agreement with the results of Edwards and Lofty (1969). The declines in earthworm community often reported in conventionally tilled soils are associated with undesirable changes in the soil environmental
535 conditions resulting from excessive tillage. However, the way in which the two tillage systems (CT and RT) are applied is of great importance. Indeed, in similar soil types (Luvisol), soil organisms in general, and earthworms in particular, are mostly (more than 90% of the individuals) found in the topsoil, the first ten centimeters; Coulibaly & Chauvat unpubl). Thus, RT system will result in similar damages performed to earthworms as in CT
540 system. The lack of detailed descriptions of tillage practices is also to blame for the contrasting findings reported by the different authors (Chan, 2001). Adoption of conservation tillage does not automatically result in an optimal earthworm community in terms of density and diversity. It may also depend from abiotic factors (Briones and Schmidt, 2017).

Another interesting aspect that contributes to the conflicting picture of the effects of
545 tillage on earthworms or Collembola is the land-use history, that is for how many years the soil has been under CT system before conversion to reduced or no-tillage system. This is important because the ability of faunal communities to adapt to a soil that has been ploughed once or several times every year is expected to increase over time, as the more resilient species will remain in the community. Therefore, soon after land-use conversion, mortality
550 rates are expected to be higher than in those soils that have been ploughed for many years, but food supply can also be temporarily higher (Detwiler, 1986), and individual fields will differ in their suitability for recolonization by soil fauna from field margins (Roarty and Schmidt,

2013). For example, Briones and Schmidt (Briones and Schmidt, 2017) have shown in their meta-analysis a very strong positive response of earthworm populations to RT operations when the soil had been under RT for a long time (>10 years). The available literature offers contrasting responses, ranging from prompt recoveries after a few months to soil fauna numbers remaining very low after many years under conventional tillage (more 15 years), but also either increases and decreases at time intervals of just a few weeks.

4.3. Relationships between soil fauna, physico-chemical parameters and microflora

Unlike Collembola assemblages, the earthworms did not recover in the three tillage systems before harvest (D210), showing progressive decreases in density and diversity since D30. The fact that the two groups of soil organisms have different life cycles could explain this (as indicated in our second hypothesis). As also stipulated in the introduction, earthworms and Collembola are of different size and may thus be differentially impaired by the machinery used for tillage. Sensitivity to herbicides may also be another explanation for differential responses of earthworms and Collembola. While Collembola often have a hydrophobic chitin cuticle (Gundersen et al., 2014; Hensel et al., 2016; Nickerl et al., 2014), the earthworm epidermis allows toxin to diffuse through (Laverack, 2013). Moreover, Collembola are known to be linked to the root system as a hot spot of microbial activity. They are known to be rather controlled by bottom-up resources (Henneron et al., 2016; Schneider and Maraun, 2009). Thus, we suggest that maize root growth after D56 has promoted microbial community in their vicinities and consequently stimulated Collembola assemblages. However, on the other hand, it is known that root growth attenuates at anthesis (flowering), when the (annual) crop transitions into seed set which replaces roots as the sink for photosynthates. The results of our RDA supported our suggestion with microbial biomass carbon (MBC), SOC stock and bulk density (BD) showing stronger links with the density and diversity of Collembola when moving away from tillage dates or in the absence of mechanical disturbance. A more in deep

study of the rhizosphere would be needed to unravel determinants of Collembola abundance and diversity changes across the maize phenological stages.

580 Finally, in agricultural soils, earthworms affect many soil properties, such as soil structure, dynamics of organic matter (Brown et al., 2004), and can be very sensitive to variations in texture and pH. This is probably the case of our study, because by focusing on our RDA results, total density and species richness earthworms were linked to MWD. These links were stronger when moving away from the tillage dates of the soil or in total absence of tillage.

585 Frey et al., (1999) showed that the mean weight diameter (MWD) of water-stable aggregates decreased significantly with the increase in tillage intensity. Finally, among the three RDA results (CT, RT, NT treatments), the highest values R² values and relative axis inertia were found for the CT treatment. This could imply the strongest statistical link biology – physicochemical parameters. As said earlier, all our experimental plots were prior to our

590 experiment managed under CT for at least 20 years. Thus, shifting from CT towards less intensive management practices (by minimizing mechanical disturbances) may produce at a short time scale a deterioration of the links between biology-environment-practice previously established. This, till a novel equilibrium may be reached for each new system implemented. The time needed to reach an equilibrium may depend several parameters like the new practice

595 applied, the organisms considered or the land-use history.

5. Conclusion

This study along a vegetative season allowed us to follow the dynamic of collembolans, earthworms, physico-chemical parameters and microflora in three levels of tillage on one site, which has historically been managed under intensive conventional tillage for at least 20 years.

600 Our findings showed that the stopping of tillage has led to an increase in the density and diversity of earthworms and Collembola over time. This change in the soil compartment is

related to the improvement of some soil conditions such as basic food source (microbial biomass of carbon, stock of soil organic carbon) and the living space/physical structure (bulk density, MWD, aggregates size). Furthermore, assemblages of earthworms and Collembola
605 do not show the same response to the three systems of tillage over time. The differences occur in the Collembola assemblages only after the repetition of the mechanical disturbance, whereas earthworms responded already after the first tillage operation. This suggests a greater resistance of smaller organisms to the effect of tillage compared to larger ones. Furthermore, during our experiment, Collembola assemblages were more resilient than assemblages of
610 earthworms probably due to the role of external driving factors like climate or vegetation growing. The variability observed between sampling dates, namely the fact that the interaction between sampling date and tillage treatment were significant for many parameters, highlights the importance of multiple samplings in time to ascertain robust conclusions on mechanical management effects upon edaphic organisms and their associated functions.

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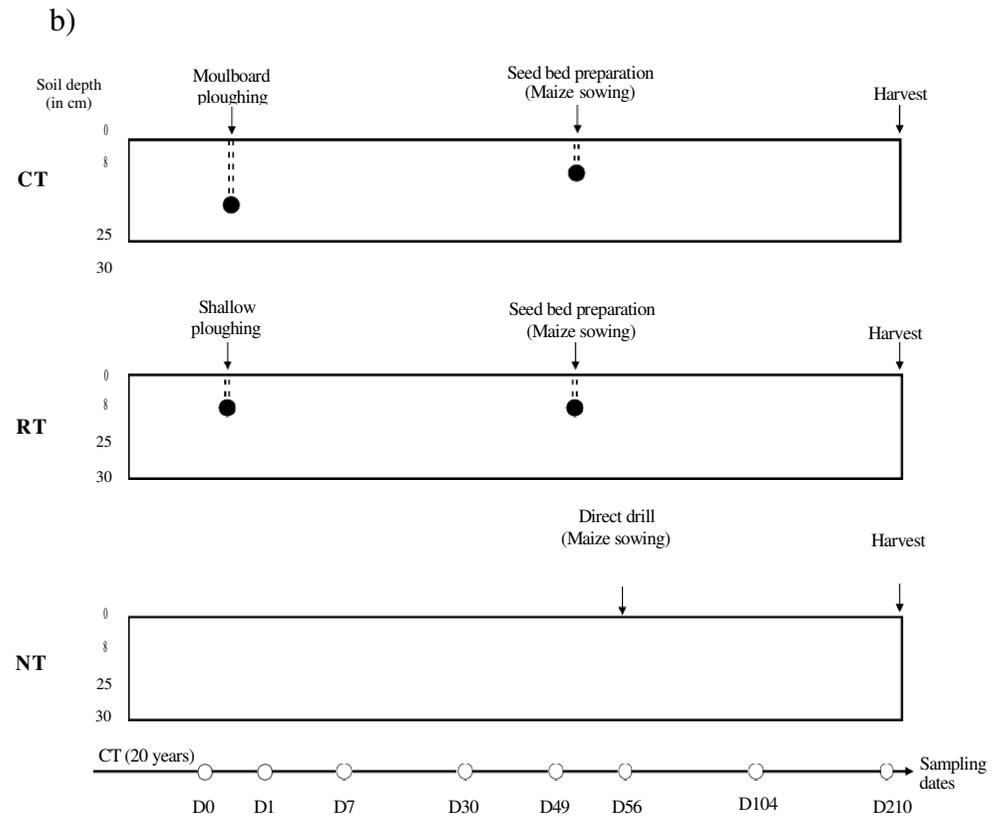
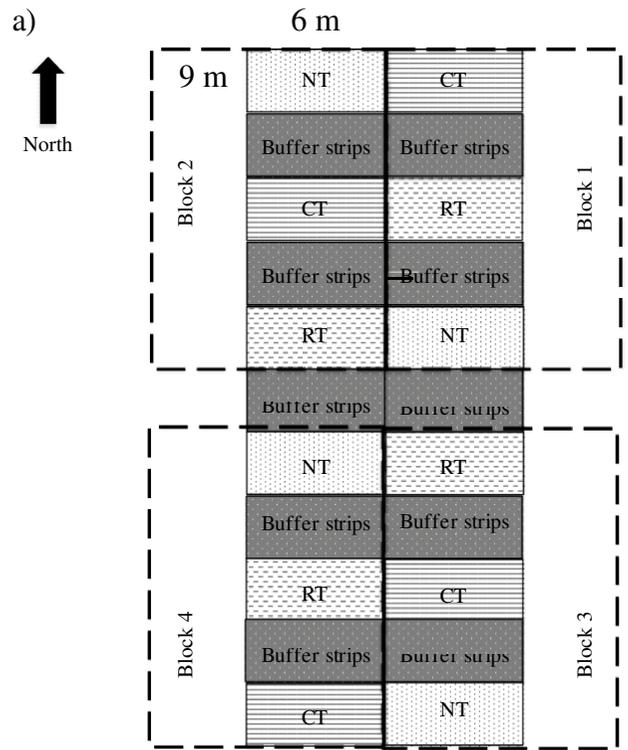


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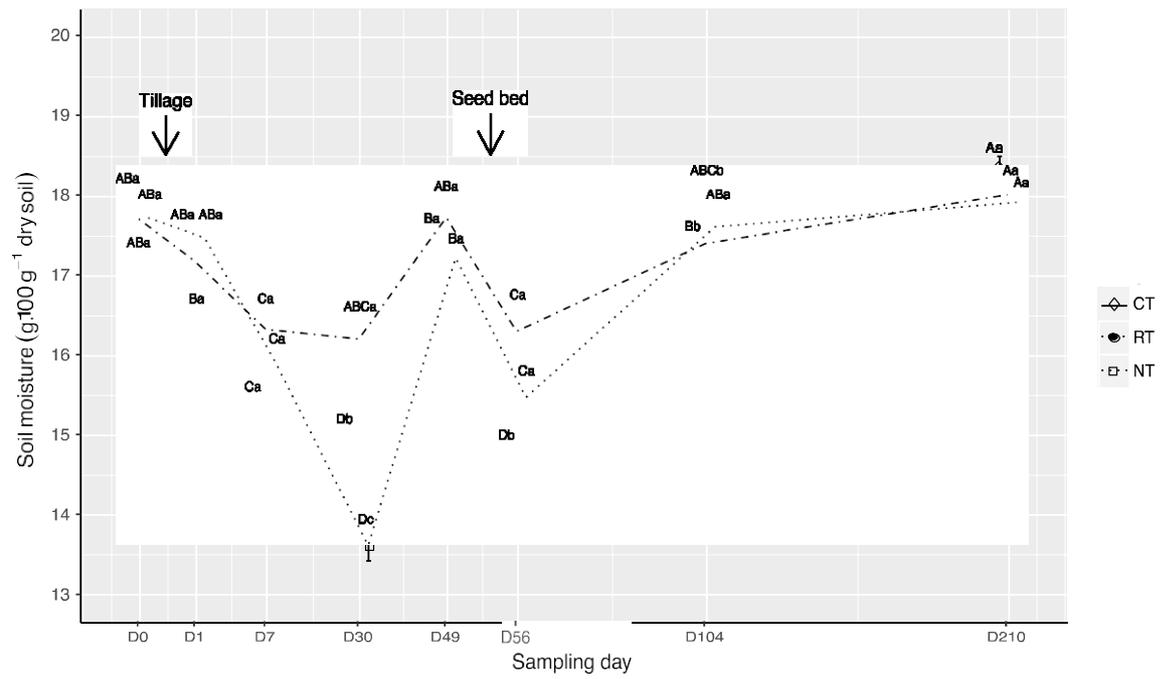


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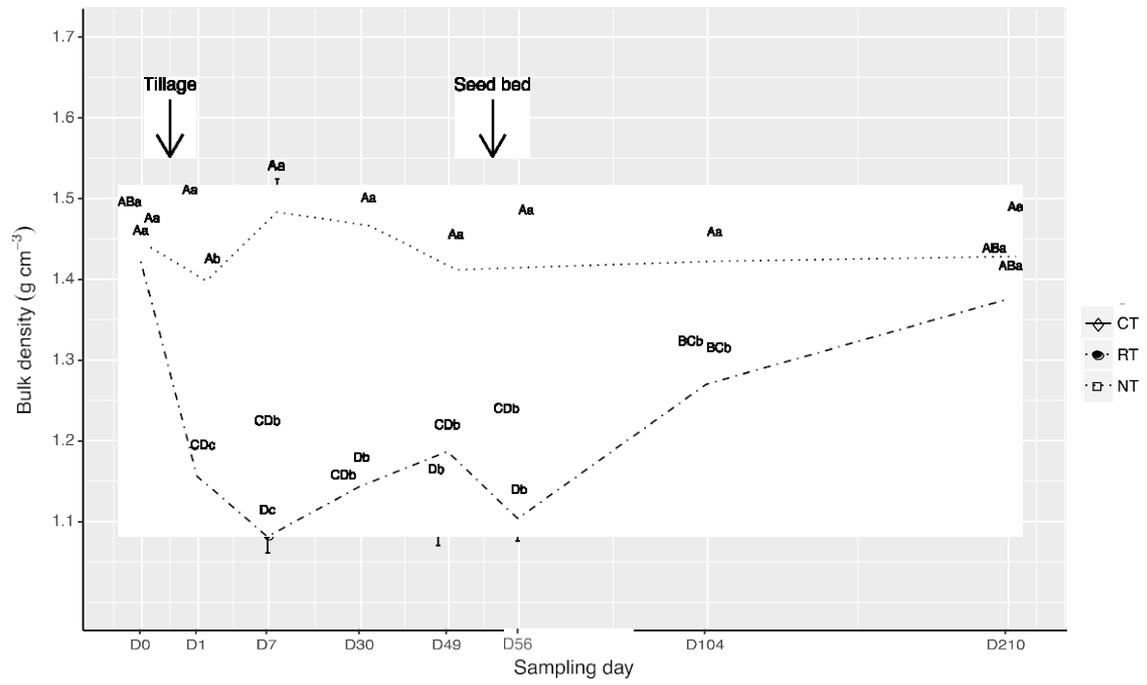


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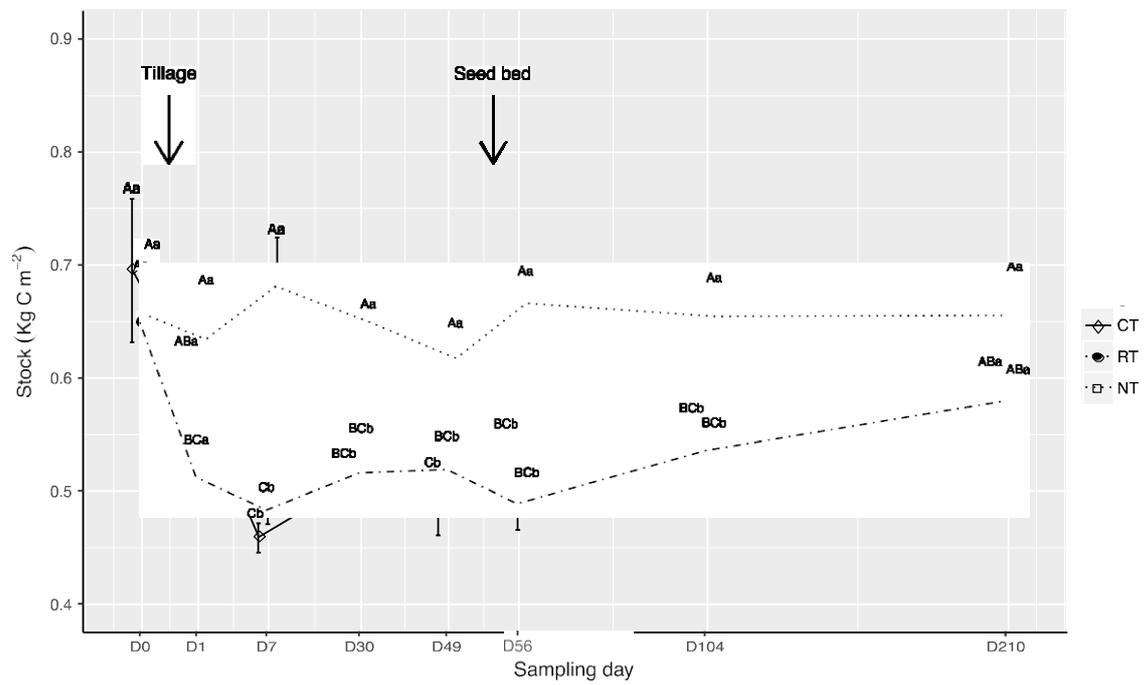


Figure 4

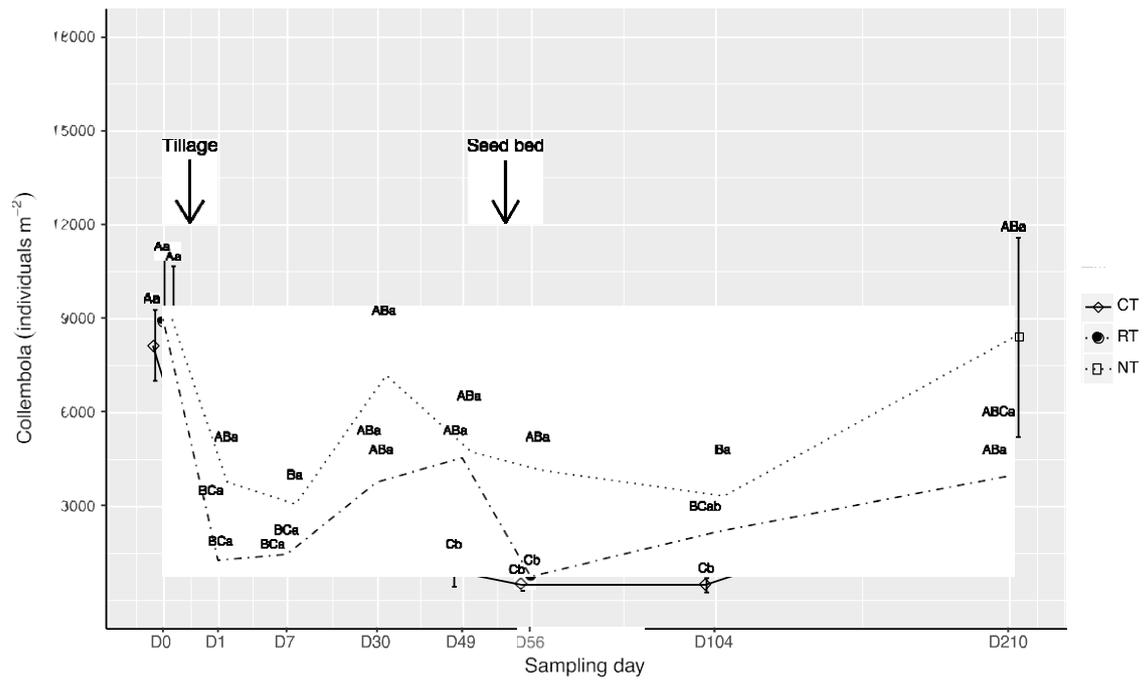


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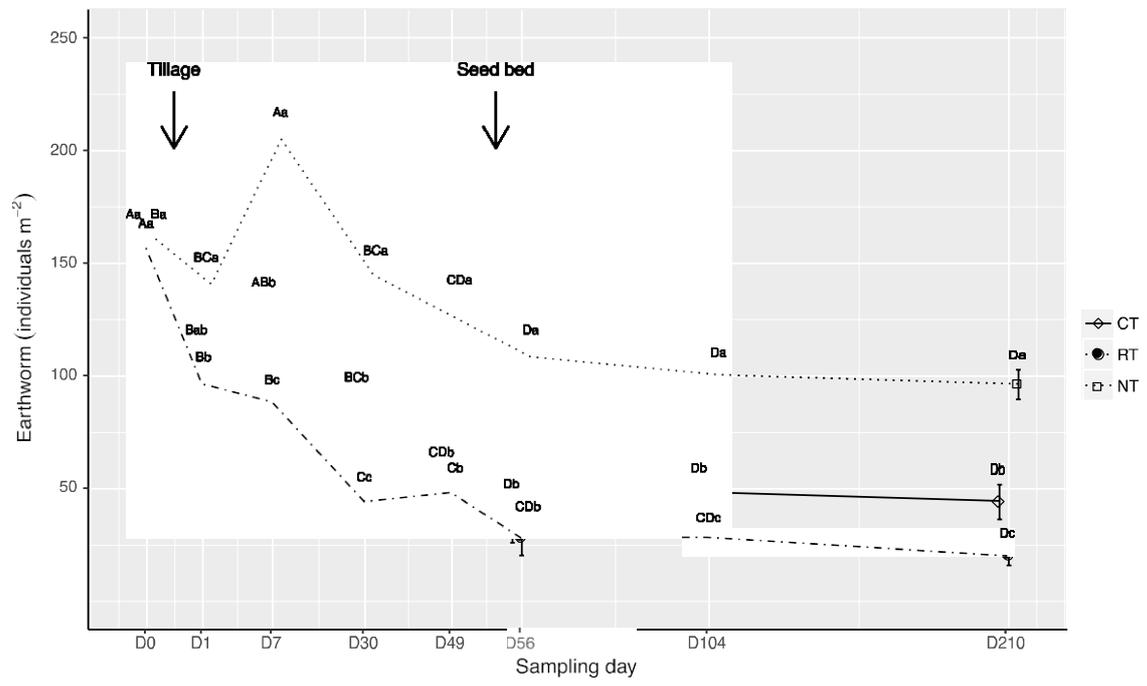


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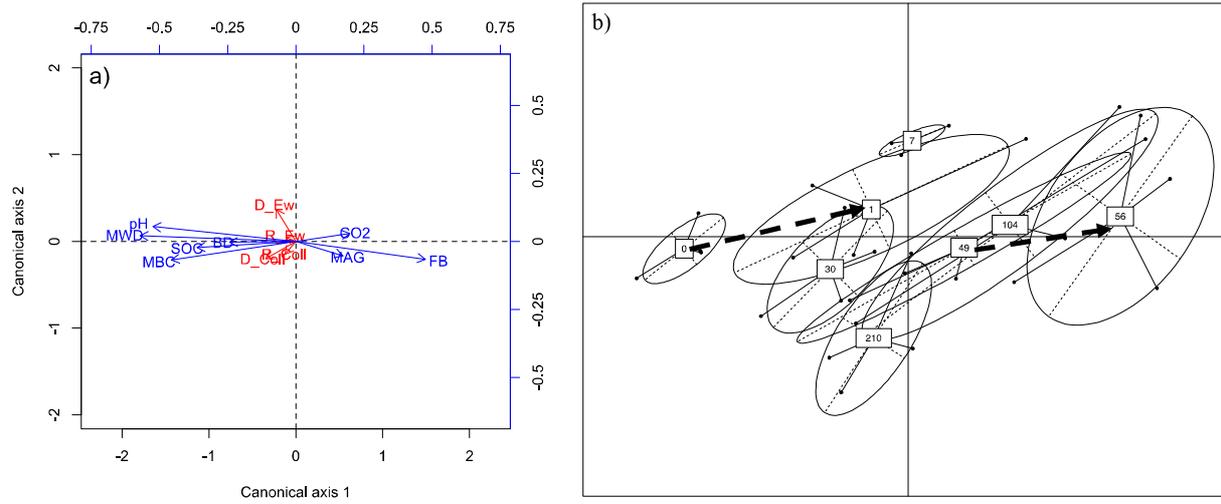


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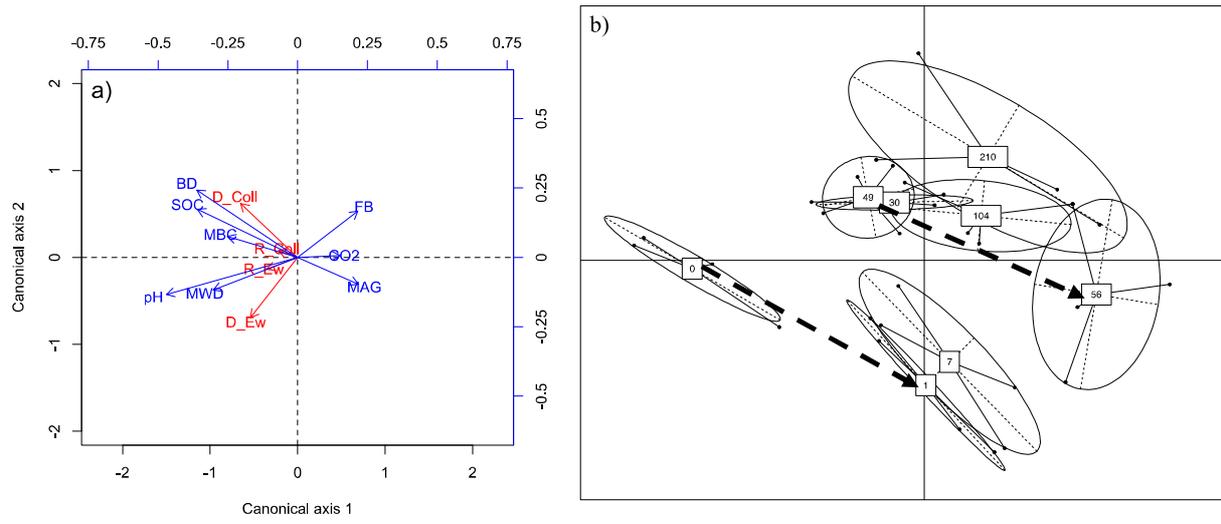


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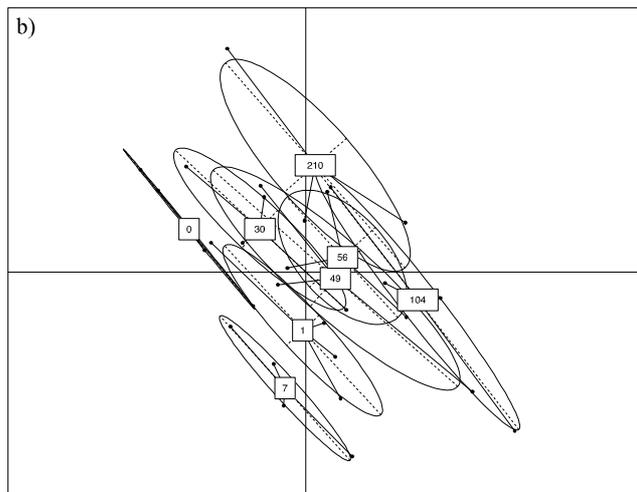
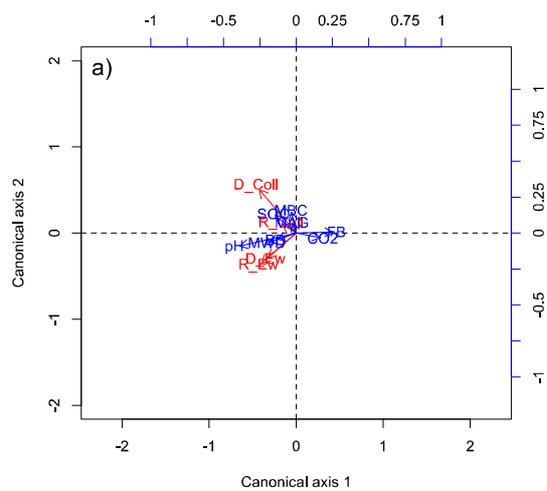


Figure 9 :

Figure captions:

Figure 1: a) Design of the experiment set-up and samplings schedule. 12 sub-plots in total, each sub-plot is 9 m long and 6 m wide; CT = Conventional Tillage, RT = Reduced Tillage, NT = No Tillage. b) The dotted lines represent soil tillage depth. Samplings: 0: 1 day before Tillage, 1: 1 day after Tillage (aT), 2: 7 days aT, 3: 30 days aT, 4: 49 days aT, 5: 56 days aT, 6: 104 days aT, 7: 210 days aT.

Figure 2: Soil moisture on 0-5 cm in depth (means and standard error) of three tillage treatments (CT: Conventional tillage, RT: Reduced tillage, NT: No-tillage) over a 8 sampling dates period (D0: 1 day before tillage, and respectively D1: 1; D7: 7; D30: 30; D49: 49; D56: 56; D104: 104; D210: 210 days after tillage) performed in Estrées-Mons, Picardie, France. For each sampling date, different minuscule letters (i.e. “a”, “b”) indicated significant differences ($\alpha = 0.05$) between treatments. Different capital letters (i.e. “A”, “B”) showed significant differences ($\alpha = 0.05$) between sampling dates within a single tillage treatment.

Figure 3: Soil bulk density on 0-5 cm in depth (means and standard error) of three tillage treatments (CT: Conventional tillage, RT: Reduced tillage, NT: No-tillage) over a 8 sampling dates period (D0: 1 day before tillage, and respectively D1: 1; D7: 7; D30: 30; D49: 49; D56: 56; D104: 104; D210: 210 days after tillage) performed in Estrées-Mons, Picardie, France. For each sampling date, different minuscule letters (i.e. “a”, “b”) indicated significant differences ($\alpha = 0.05$) between treatments. Different capital letters (i.e. “A”, “B”) showed significant differences ($\alpha = 0.05$) between sampling dates within a single tillage treatment.

Figure 4: SOC stock on 0- to 5-m in depth (means and standard error) of three tillage treatments (CT: Conventional tillage, RT: Reduced tillage, NT: No-tillage) over a 8 sampling dates period (D0: 1 day before tillage, and respectively D1: 1; D7: 7; D30: 30; D49: 49; D56: 56; D104: 104; D210: 210 days after tillage) performed in Estrées-Mons, Picardie, France. For each sampling date, different minuscule letters (i.e. “a”, “b”) indicated significant differences ($\alpha = 0.05$) between treatments. Different capital letters (i.e. “A”, “B”) showed significant differences ($\alpha = 0.05$) between sampling dates within a single tillage treatment.

Figure 5: Total density of Collembola assemblages (means and standard error) of three tillage treatments (CT: Conventional tillage, RT: Reduced tillage, NT: No-tillage) over a 8 sampling dates period (D0: 1 day before tillage, and respectively D1: 1; D7: 7; D30: 30; D49: 49; D56:

56; D104: 104; D210: 210 days after tillage) performed in Estrées-Mons, Picardie, France. For each sampling date, different minuscule letters (i.e. “a”, “b”) indicated significant differences ($\alpha = 0.05$) between treatments. Different capital letters (i.e. “A”, “B”) showed significant differences ($\alpha = 0.05$) between sampling dates within a single tillage treatment.

Figure 6: Total density of earthworms (means and standard error) of three tillage treatments (CT: Conventional tillage, RT: Reduced tillage, NT: No-tillage) over a 8 sampling dates period (D0: 1 day before tillage, and respectively D1: 1; D7: 7; D30: 30; D49: 49; D56: 56; D104: 104; D210: 210 days after tillage) performed in Estrées-Mons, Picardie, France. For each sampling date, different minuscule letters (i.e. “a”, “b”) indicated significant differences ($\alpha = 0.05$) between treatments. Different capital letters (i.e. “A”, “B”) showed significant differences ($\alpha = 0.05$) between sampling dates within a single tillage treatment.

Figure 7: Redundancy analysis (RDA) biplot of the conventional tillage (CT) data showing : a) soil fauna variables (D_Ew : Earthworms density; R_Ew : Earthworms species richness; D_Coll: Collembola density; R_Coll : Collembola species richness; MBC : Microbial biomass carbon; FB : Fungal biomass), environmental variables (SM: Soil moisture; pH: pH_{water}; CO2: potential C mineralisation; SOC : SOC stock on 0- to 5-m; BD: Bulk density 0-5 cm; MWD : Mean weight diameter; MAG : Macro-aggregates or aggregates > 250 μ m) and b) plotting sampling days (0 : 1 day before tillage; 1; 7; 30 ; 49; 56; 104; 210 respectively after tillage).

Figure 8: Redundancy analysis (RDA) biplot of the reduced tillage (RT) data showing : a) soil fauna variables (D_Ew : Earthworms density; R_Ew : Earthworms species richness; D_Coll: Collembola density; R_Coll : Collembola species richness; MBC : Microbial biomass carbon; FB : Fungal biomass), environmental variables (SM: Soil moisture; pH: pH_{water}; CO2: potential C mineralisation; SOC : SOC stock on 0- to 5-m; BD: Bulk density 0-5 cm; MWD : Mean weight diameter; MAG : Macro-aggregates or aggregates > 250 μ m) and b) plotting sampling days (0 : 1 day before tillage; 1; 7; 30 ; 49; 56; 104; 210 respectively after tillage).

Figure 9: Redundancy analysis (RDA) biplot of the no tillage (NT) data showing : a) soil fauna variables (D_Ew : Earthworms density; R_Ew : Earthworms species richness; D_Coll: Collembola density; R_Coll : Collembola species richness; MBC : Microbial biomass carbon; FB : Fungal biomass), environmental variables (SM: Soil moisture; pH: pH_{water}; CO2: potential C mineralisation; SOC : SOC stock on 0- to 5-m; BD: Bulk density 0-5 cm; MWD : Mean

weight diameter; MAG : Macro-aggregates or aggregates $> 250 \mu\text{m}$) and b) plotting sampling days (0 : 1 day before tillage; 1; 7; 30 ; 49; 56; 104; 210 respectively after tillage).

Table 1: Results of Linear Mixed-Effects Models (LMM) of “the effect of treatment for each sampling date” and “the effect of sampling day for each treatment” on Soil moisture, bulk density (0- to 5-cm) and SOC stock (0- to 0.05-m). D0: 1 day before tillage, and respectively D1: 1; D7: 7; D30: 30; D49: 49; D56: 56; D104: 104; D210: 210 days after tillage. CT: Conventional tillage, RT: Reduced tillage and NT: No tillage. The tests values are given. F: F- value, p: p-value, R2: variance explained by both fixed and random factors. The rejection level was set at $\alpha = 0.05$. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, ns $p > 0.05$.

Table 2: Soil Aggregate mean weight diameter (MWD) (means \pm standard errors) of three different tillage treatments (CT: Conventional tillage, RT: Reduced tillage, NT: No-tillage) over a 8 sampling dates period (D0: 1 day before tillage, and respectively D1: 1; D7: 7; D30: 30; D49: 49; D56: 56; D104: 104; D210: 210 days after tillage) performed in Estrées-Mons, Picardie, France. For each sampling date, different minuscule letters (i.e. “a”, “b”) indicated significant differences ($\alpha = 0.05$) between treatments. Different capital letters (i.e. “A”, “B”) showed significant differences ($\alpha = 0.05$) between sampling dates for each experimental treatment.

Table 3: Soil microbial biomass carbon (MBC) and fungal biomass (FB) (means \pm standard errors) of three different tillage treatments (CT: Conventional tillage, RT: Reduced tillage, NT: No-tillage) over a 8 sampling date period (D0: 1 day before tillage, and respectively D1: 1; D7: 7; D30: 30; D49: 49; D56: 56; D104: 104; D210: 210 days after tillage) performed in Estrées- Mons, Picardie, France. For each sampling date, different minuscule letters (i.e. “a”, “b”) indicated significant differences ($\alpha = 0.05$) between treatments. Different capital letters (i.e. “A”, “B”) showed significant differences ($\alpha = 0.05$) between sampling dates for each experimental treatment.

Table 4: C-CO₂ (means \pm standard errors) of three different tillage treatments (CT: Conventional tillage, RT: Reduced tillage, NT: No-tillage) over a 8 sampling date period (D0: 1 day before tillage, and respectively D1: 1; D7: 7; D30: 30; D49: 49; D56: 56; D104: 104; D210: 210 days after tillage) performed in Estrées-Mons, Picardie, France. For each sampling date, different minuscule letters (i.e. “a”, “b”) indicated significant differences ($\alpha = 0.05$) between treatments. Different capital letters (i.e. “A”, “B”) showed significant differences ($\alpha = 0.05$) between sampling dates for each experimental treatment.

Table 5: Results of Linear Mixed-Effects Models (LMM) of “the effect of treatment for each sampling date” and “the effect of sampling day for each treatment” on total density, species richness and Shannon index of Collembola and earthworms. D0: 1 day before tillage, and respectively D1: 1; D7: 7; D30: 30; D49: 49; D56: 56; D104: 104; D210: 210 days after tillage. CT: Conventional tillage, RT: Reduced tillage and NT: No tillage. The tests values are given. F: F-value, p: p-value, R²: variance explained by both fixed and random factors. The rejection level was set at $\alpha = 0.05$. ***p<0.001, **p<0.01, *p<0.05, ns p>0.05.

Table 6: Results of redundancy analysis of three treatments (CT: Conventional tillage, RT: Reduced tillage, NT: No-tillage) based on tests of permutations (n=999). The values of the first two axis are given.

Table 1:

	Tillage effect								
	Soil moisture			Bulk density			Stock C		
	<i>F</i>	<i>p</i>	<i>R</i> ² (%)	<i>F</i>	<i>p</i>	<i>R</i> ² (%)	<i>F</i>	<i>p</i>	<i>R</i> ² (%)
D0	0.51	ns	8.54	0.13	ns	5.93	0.25	ns	5.37
D1	0.45	ns	8.73	107	***	95.1	3.95	ns	41.8
D7	0.90	ns	14.0	53.0	***	90.6	18.0	**	76.6
D30	8.23	*	60.6	70.6	***	92.8	15.2	**	73.4
D49	1.23	ns	20.0	25.3	**	82.1	12.6	**	71.7
D56	11.8	**	71.8	17.4	**	76.8	14.2	**	72.1
D104	0.13	ns	2.40	8.45	*	60.8	11.7	**	68.8
D210	2.28	ns	29.3	0.86	ns	50.1	3.36	ns	41.6
	Sampling day effect								
	Soil moisture			Bulk density			Stock C		
	<i>F</i>	<i>p</i>	<i>R</i> ² (%)	<i>F</i>	<i>p</i>	<i>R</i> ² (%)	<i>F</i>	<i>p</i>	<i>R</i> ² (%)
CT	40.5	***	90.2	13.5	***	75.3	11.7	***	76.4
RT	12.8	*	22.5	22.0	***	83.2	6.02	***	60.8
NT	102	***	96.0	0.64	ns	12.6	0.67	ns	55.1

Table 2:

Physico-chemical properties						
MWD (mm)						
Sampling day	CT	RT	NT	<i>F</i>	<i>p-value</i>	<i>R2</i>
D0	3.27 ± 0.06 ^{A a}	3.27 ± 0.09 ^{A a}	3.19 ± 0.06 ^{A a}	0.34	ns	5.78
D1	2.60 ± 0.05 ^{BC a}	3.04 ± 0.23 ^{AB a}	3.16 ± 0.17 ^{A a}	3.17	ns	36.6
D7	2.88 ± 0.13 ^{AB a}	3.32 ± 0.10 ^{A a}	3.21 ± 0.12 ^{A a}	4.78	ns	54.4
D30	2.65 ± 0.13 ^{BC b}	2.88 ± 0.07 ^{ABC b}	3.16 ± 0.10 ^{A a}	11.4	**	74.5
D49	2.67 ± 0.12 ^{BC a}	2.59 ± 0.19 ^{BCD a}	2.92 ± 0.19 ^{A a}	1.41	ns	41.2
D56	2.27 ± 0.12 ^{CD b}	2.54 ± 0.13 ^{CD ab}	2.77 ± 0.09 ^{A a}	5.87	*	57.9
D104	2.17 ± 0.11 ^{D b}	2.42 ± 0.08 ^{D b}	2.98 ± 0.10 ^{A a}	18.6	**	77.2
D210	2.73 ± 0.08 ^{B a}	2.99 ± 0.11 ^{ABC a}	3.01 ± 0.12 ^{A a}	2.05	ns	27.2
LMM outputs						
<i>F</i>	12.9	8.85	1.67	-	-	-
<i>p-value</i>	***	***	ns	-	-	-
<i>R2</i>	76.1	71.4	28.5	-	-	-

Table 3:

Sampling day	Microbial communities											
	MBC ($\mu\text{g g}^{-1}$)			LMM outputs			Fungal biomass ($\mu\text{g g}^{-1}$)			LMM outputs		
	CT	RT	NT	<i>F</i>	<i>p-value</i>	<i>R</i> ²	CT	RT	NT	<i>F</i>	<i>p-value</i>	<i>R</i> ² (%)
D0	408 ± 9.78 ^{AB a}	418 ± 9.20 ^{B a}	428 ± 11.6 ^{AB a}	0.97	ns	18.9	1.70 ± 0.23 ^{BC a}	1.81 ± 0.28 ^{BC a}	1.80 ± 0.24 ^{CD a}	0.27	ns	78.4
D1	380 ± 28.3 ^{ABC b}	505 ± 13.8 ^{A a}	455 ± 24.8 ^{A ab}	7.51	*	57.7	1.83 ± 0.07 ^{BC b}	2.15 ± 0.06 ^{B a}	1.95 ± 0.03 ^{CD b}	8.03	*	59.4
D7	342 ± 21.0 ^{BC a}	236 ± 21.0 ^{D b}	217 ± 22.7 ^{D b}	10.4	*	66.2	1.51 ± 0.04 ^{C a}	1.73 ± 0.03 ^{C a}	1.71 ± 0.15 ^{CD a}	1.69	ns	23.5
D30	300 ± 18.9 ^{CD b}	359 ± 6.48 ^{Ca}	286 ± 16.7 ^{CD b}	7.21	*	58.6	1.68 ± 0.13 ^{BC ab}	1.86 ± 0.04 ^{C a}	1.63 ± 0.06 ^{D b}	3.85	*	62.3
D49	322 ± 26.3 ^{C b}	413 ± 23.3 ^{BC a}	440 ± 22.4 ^{A a}	10.8	*	72.2	2.33 ± 0.17 ^{AB b}	3.45 ± 0.24 ^{A a}	3.70 ± 0.21 ^{A a}	14.3	**	73.6
D56	223 ± 18.1 ^{D a}	282 ± 35.6 ^{CD a}	259 ± 21.0 ^{CD a}	3.09	ns	66.4	2.79 ± 0.14 ^{A a}	2.81 ± 0.16 ^{A a}	2.85 ± 0.18 ^{AB a}	0.05	ns	10.5
D104	343 ± 16.2 ^{BC a}	389 ± 27.9 ^{BC a}	341 ± 30.7 ^{BC a}	2.21	ns	57.3	2.47 ± 0.07 ^{A b}	3.21 ± 0.12 ^{A a}	2.74 ± 0.17 ^{B ab}	9.31	*	63.6
D210	428 ± 13.3 ^{A a}	398 ± 12.8 ^{BC a}	428 ± 14.8 ^{AB a}	1.57	ns	22.2	2.13 ± 0.24 ^{AB a}	2.20 ± 0.21 ^{BC a}	1.93 ± 0.06 ^{CD a}	0.71	ns	24.2
LMM outputs												
<i>F</i>	10.7	16.0	20.5	-	-	-	8.29	25.4	21.5	-	-	-
<i>p-value</i>	***	***	***	-	-	-	***	***	***	-	-	-
<i>R</i> ² (%)	70.6	78.3	82.5	-	-	-	65.77	86.3	82.9	-	-	-

Table 4:

Sampling day	Potential C mineralisation (mg kg ⁻¹ d ⁻¹)			LMM outputs		
	C-CO ₂			<i>F</i>	<i>p-value</i>	<i>R</i> ² (%)
	CT	RT	NT			
D0	21.2 ÷ 0.71 ^{B a}	20.8 ÷ 1.16 ^{D a}	22.6 ÷ 1.72 ^{C a}	1.02	ns	53.1
D1	25.6 ± 1.38 ^{A a}	27.2 ± 1.44 ^{BC a}	23.0 ± 1.07 ^{C a}	2.55	ns	31.7
D7	21.1 ÷ 0.67 ^{B b}	25.6 ÷ 0.76 ^{C a}	24.3 ÷ 1.13 ^{BC ab}	6.77	*	55.2
D30	23.2 ± 0.22 ^{AB b}	25.9 ± 0.44 ^{BC a}	24.2 ± 0.55 ^{BC b}	9.81	*	64.1
D49	23.3 ÷ 0.91 ^{AB b}	29.4 ÷ 0.52 ^{AB a}	29.0 ÷ 0.71 ^{A a}	21.3	**	79.5
D56	22.6 ± 0.64 ^{AB a}	23.1 ± 0.56 ^{CD a}	24.1 ± 0.48 ^{BC a}	3.39	ns	62.5
D104	24.2 ÷ 0.43 ^{AB c}	30.9 ÷ 0.32 ^{A a}	26.7 ÷ 0.60 ^{AB b}	47.3	***	89.6
D210	22.4 ± 0.73 ^{AB a}	22.4 ± 0.57 ^{D a}	22.7 ± 0.04 ^{C a}	0.10	ns	1.70
LMM outputs						
<i>F</i>	3.66	19.9	8.89	-	-	-
<i>p-value</i>	**	***	***	-	-	-
<i>R</i> ² (%)	45.2	82.4	71.8	-	-	-

Table 5:

	Tillage effect																	
	Collembola									Earthworm								
	Density			Species richness			Shannon index			Density			Species richness			Shannon index		
	<i>F</i>	<i>p</i>	R ² (%)	<i>F</i>	<i>p</i>	R ² (%)	<i>F</i>	<i>p</i>	R ² (%)	<i>F</i>	<i>p</i>	R ² (%)	<i>F</i>	<i>p</i>	R ² (%)	<i>F</i>	<i>p</i>	R ² (%)
D0	0.04	ns	26.8	0.27	ns	24.8	0.39	ns	0.66	0.12	ns	12.2	0.14	ns	2.42	0.16	ns	2.85
D1	2.51	ns	31.4	1.10	ns	16.7	0.65	ns	27.1	5.29	*	49.0	8.6	*	61.0	14.1	**	71.9
D7	4.78	ns	50.2	1.53	ns	47.9	1.09	ns	26.3	123	***	95.7	36.7	***	86.7	15.1	**	73.2
D30	2.34	ns	33.0	3.89	ns	54.5	3.84	ns	62.5	32.4	***	85.5	9.00	*	62.1	8.59	*	62.1
D49	6.59	*	54.5	3.89	ns	54.5	4.34	ns	67.7	22.5	**	80.4	19.5	**	78.6	17.4	**	77.7
D56	25.9	***	82.5	34.2	***	86.5	13.8	**	73.6	18.4	**	77.0	14.33	**	76.6	6.95	*	62.8
D104	6.32	*	55.9	6.77	*	55.2	10.3	**	65.2	39.8	**	87.8	4.20	ns	63.8	2.08	ns	60.7
D210	1.73	ns	24.0	0.65	ns	10.6	0.79	ns	12.6	55.1	***	91.3	4.50	ns	48.5	3.99	ns	43.3
	Sampling day effect																	
	Collembola									Earthworm								
	Density			Species richness			Shannon index			Density			Species richness			Shannon index		
	<i>F</i>	<i>p</i>	R ² (%)	<i>F</i>	<i>p</i>	R ² (%)	<i>F</i>	<i>p</i>	R ² (%)	<i>F</i>	<i>p</i>	R ² (%)	<i>F</i>	<i>p</i>	R ² (%)	<i>F</i>	<i>p</i>	R ² (%)
CT	11.7	***	72.5	4.40	***	49.8	3.12	*	41.3	33.4	***	89.0	17.8	***	80.7	9.82	***	68.9
RT	7.34	***	62.4	4.07	**	55.1	2.48	*	51.9	38.0	***	89.6	18.1	***	80.4	8.51	***	65.8
NT	2.77	*	45.8	2.47	*	37.4	1.84	*	35.5	22.4	***	84.0	19.6	***	81.7	13.4	***	75.9

Table 6:

	Redundancy statistic			Relative inertia	
	F	p-value	R ² (%)	axis 1 (%)	axis 2 (%)
CT	3.04	0.009	37.2	91.1	6.67
RT	1.88	0.037	20.3	53.1	21.8
NT	0.99	0.49	2.71	20.0	6.01