



HAL
open science

Beneficial effects of conservation agriculture on earthworm and Collembola communities in Northern France

Anne-Maïmiti Dulaurent, David Houben, Nicolas Honvault, Michel-Pierre Faucon, Matthieu Chauvat

► **To cite this version:**

Anne-Maïmiti Dulaurent, David Houben, Nicolas Honvault, Michel-Pierre Faucon, Matthieu Chauvat. Beneficial effects of conservation agriculture on earthworm and Collembola communities in Northern France. *Plant and Soil*, In press, 10.1007/s11104-023-05916-9. hal-04027974

HAL Id: hal-04027974

<https://normandie-univ.hal.science/hal-04027974>

Submitted on 22 Mar 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Beneficial effects of conservation agriculture on earthworm and Collembola communities in**

2 **Northern France**

3

4 Anne-Maïmiti Dulaurent¹, David Houben¹, Nicolas Honvault¹, Michel-Pierre Faucon^{1*} & Matthieu Chauvat^{2*}

5

6 1 UniLaSalle, AGHYLE, UP.2018.C101, FR-60026 Beauvais, France, SFR Condorcet FR CNRS 3417

7 2 Normandie Université, UNIROUEN, INRAE, ECODIV, Rouen, France

8 * These authors contributed equally to this work

9 Correspondance : A.-M. Dulaurent (corresponding author), UniLaSalle, AGHYLE, UP.2018.C101, 19 Rue Pierre

10 Waguët, F-60026 Beauvais, France. Tel: + 33 – (0)3.44.06.38.60

11 Fax: + 33 - (0)3.44.06.25.26. E-mail : anne-maimiti.dulaurent@unilasalle.fr

12

13 **Acknowledgments**

14 The project was partly funded by the Association pour la Promotion d'une Agriculture Durable (APAD). The authors

15 thank the farmers for the access to their farm and their technical assistance. We also thank the students of the 156th

16 class ASET of UniLaSalle, Guy Josens and Elodie Fouché for their technical assistance. The authors thank Thomas

17 Damestoy for his pre-submission review of the manuscript, as well as Romain Gloaguen and three anonymous

18 reviewers for their useful comments to improve the quality of the manuscript.

19

20 **Abstract:**

21 *Aim* Conservation agriculture is increasingly adopted to improve soil fertility and reduce the depressive effects of

22 intensive farming on soil functions and associated services. However, conservation agriculture systems may vary

23 greatly regarding time since conversion to this system and the quality and biomass production of cover crops. In

24 addition, the effects of these variables on soil fauna are still poorly known. Here, we aim to gain insight into the

25 impact of conservation agriculture on soil mesofauna and macrofauna communities at the cropping system level.

26 *Method* Collembola and earthworm communities were compared in seven neighbouring pairs of fields (same

27 topography, spring crop and soil type) being either under conventional systems or under conservation agriculture

28 systems with cover crops.

29 *Results* Conservation agriculture systems influenced soil mesofauna and macrofauna communities compared to
30 conventional systems. The beneficial effects of conservation agriculture on earthworm communities may
31 predominantly be related to the lower soil physical disturbance (no-tillage) while Collembola may also be positively
32 affected by cover crops characteristics and time since conversion to conservation agriculture.

33 *Conclusions* The adoption of conservation agriculture systems may improve earthworm and Collembola abundance
34 and represents thus an innovative opportunity for sustainable crop production through the preservation of soil fauna.
35 Finally, we suggest the use of a trait-based approach to draw functional consequences of soil faunal community
36 changes operating in conservation agriculture.

37

38 **Keywords:** Collembola, earthworms, direct seeding, no-till, conventional agriculture.

39

40

41 **Introduction**

42 Soil fauna communities play a key role in agroecosystem functioning (Bardgett and van der Putten 2014; Bender and
43 van der Heijden 2015). For instance, earthworms are well known to enhance soil structure (i.e., macroporosity), root
44 penetration and nutrient availability (Blouin et al. 2013). Collembola, a well-studied and dominant microarthropod
45 group in agrosystems (Hopkin 1997), contribute to organic matter decomposition by the regulation of microbial
46 population levels (Hopkin 1997; Coulibaly et al. 2019), as well as N mineralization and soil respiration (Filser 2002).
47 Both groups (earthworms and Collembola) while sharing the same environment, differ in size, mobility, life cycle,
48 trophic position and therefore functions, making them an interesting duet to study in the framework of agroecosystem
49 functioning (Coulibaly et al. 2022).

50 The impact of soil fauna on agroecosystem functioning may be strongly mediated by agricultural practices which act
51 as environmental filters selecting species and changing community structures (Ponge et al. 2013; Coudrain et al.
52 2016). In particular, the abundance and species composition of earthworm and Collembola communities depend on
53 land use intensification (Ponge et al. 2003; Smith et al. 2008; Postma-Blaauw et al. 2010). In tilled systems,
54 earthworms (van Capelle et al. 2012; Ponge et al. 2013; Pelosi et al. 2014) as well as Collembola (Brennan et al. 2006;
55 Coulibaly et al. 2017, 2022) abundance and diversity are usually negatively affected by tillage due to the physical
56 disruption of the soil. However, Collembola response differs depending on the Collembola life-strategy and soil
57 properties (van Capelle et al. 2012), making them more resistant in the short-term to tillage than earthworms
58 (Coulibaly et al. 2022). Besides tillage, other practices like residue restitution (from cover crops or crops) may
59 positively affect earthworms (Chan 2001; Eriksen-Hamel et al. 2009; Frazão et al. 2019) and Collembola (Culik et al.
60 2002; Gatiboni et al. 2011) communities by improving soil microclimatic conditions and resource availability.
61 However, divergent results have been found among studies (Brennan et al. 2006; Scheunemann et al. 2015; Coulibaly
62 et al. 2017) and were mainly attributed to differences in crop residue quantity and quality (Sauvadet et al. 2016). In
63 addition, beneficial effects of crop residue management may be overridden by tillage practices and therefore mainly
64 be observed under no-till systems (Denier et al. 2022).

65 Association of cover crops and no-tillage is more commonly called conservation agriculture. According to the Food
66 and Agriculture Organization of the United Nations (FAO), conservation agriculture correspond to cropping systems
67 that promote (1) no-tillage or minimum soil disturbance, (2) maintenance of a permanent mulch of crop residues, (3)
68 use of productive multispecies cover crops, and (4) use of diverse and long crop rotations (Corbeels et al. 2006; Hobbs

69 2007; FAO 2022). Conservation agriculture is increasingly considered as an alternative management system that may
70 improve long-term soil fertility (Tsiafouli et al. 2015) and reduce the depressive effects of intensive farming on soil
71 functions and associated services (Holland 2004). Although the four aforementioned practices represent the pillars of
72 systems engaged in conservation agriculture, these systems may however greatly differ from each other by the
73 management of cover crops and the biomass and the quality of their residues, which may in turn impact soil fauna
74 communities and associated functions (Ghosh et al. 2019) and by the time since the system was set up.
75 With the increasing adoption of conservation agriculture practices, even in temperate areas (Houben et al. 2018), a
76 thorough understanding of the effect of the wide range of agricultural practices on soil fauna communities is essential
77 to evaluate and design innovative and sustainable cropping systems. Although many results are available at the
78 specific practice level (tillage, crop residue management, etc.), a systemic approach is needed to consider all practices
79 as a whole at the cropping system level (Henneron et al. 2015; Denier et al. 2022).

80 In the present study, we tested the effect of two contrasting agricultural systems (conventional vs. conservation
81 agriculture) on soil mesofauna (Collembola) and macrofauna (earthworms). To this end we characterized the structure
82 of soil fauna communities in plots across a real-life on-farm variability of conservation agriculture contexts and
83 practices (time since conversion, cover crops management strategies), in comparison with conventional practices,
84 under the same climate, soil context and agricultural landscape in Northern France. Overall, this study builds towards
85 a better understanding of the effects of agricultural practices on soil fauna. We hypothesized (i) that the abundance
86 and diversity of both earthworm and Collembola communities are promoted under conservation agriculture but with a
87 more contrasted response from earthworms and (ii) that soil fauna communities are structured by agricultural practices
88 and present contrasted compositions driven by the characteristics of cover crops. Especially, high biomass production
89 and quality of cover crops residues would be more favourable to fauna communities with their effects becoming more
90 visible over time.

91

92 **Methods**

93 **Site description**

94 The study was carried out in May 2016 at 14 locations within an area of 44 km² around Beauvais (Oise, France)
95 (49°25'49''N, 2°04'51''E). The region is characterised by an oceanic/temperate climate, marked by mild winters (6.5
96 °C on average), cool summers (14.9°C on average) and moderate rainfall (655 mm.year⁻¹ on average) (Météo France
97 2016). The topography is characteristic of a plateau region: vast limestone areas, overlaid by a clay layer of variable
98 thickness, at an altitude between 15 and 300 meters above sea level. Soils may also contain high proportions of silt.
99 The landscape of this region is homogeneous and mainly characterized by large areas of cropland managed under
100 conventional farming with four year-rotations focused on wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.),
101 rapeseed (*Brassica napus* L.) and pea (*Pisum sativum* L.). There are few natural elements, mainly small forests or
102 meadows close to villages.

103

104 **Sampling design and characterisation of practices variables**

105 Sites were chosen in pairs thanks to a network of farmers. After finding seven fields under conservation agriculture
106 systems (without tillage and with diversified cover crops - hereafter “CA system”), seven adjacent fields under
107 conventional cropping system (with tillage and simplified or no cover crops – hereafter “Conventional system”) were
108 selected. Conventional fields were chosen such that they had the same topography, spring crop and soil type as their
109 paired field under CA system (Table 1). All soils were loess-derived Haplic Luvisol. CA systems showed variability
110 in the time since conversion (ranging from 2 to 9 years) and the characteristics of the cover crops (biomass, species
111 richness and diversity) (Table 1). Within each site, in an homogeneous and representative part of the field, 40 meters
112 away from any border, four plots were set up along a transect, 20 meters distance from each other, for a total number
113 of 56 samples (4 plots x 7 fields x 2 farm types).

114 For each site under conservation agriculture, the time since conversion was obtained from the farmer. When they were
115 present, cover crops were characterized within four 1-m² plots by determining total biomass after 48h of drying at
116 60°C, total species richness and Simpson diversity index ($D = \sum Ni(Ni-1)/N(N-1)$, where D is Simpson diversity
117 index, Ni is the number of individuals of species i and N is the total number of individuals) (Simpson 1949). Simpson
118 index was used to characterize cover crops diversity because this kind of cultivated communities does not contain any
119 low-abundance species, as sowing density is designed by the farmer.

120

121 **Soil chemical properties**

122 In each plot, approximately 1 000 g of top soil (0 – 10 cm) were collected by composite sampling to confirm that each
123 field pair presented the same soil type. Soil samples were dried at room temperature and sieved (2 mm). pH in water
124 (NF ISO 10390), organic carbon (C), total nitrogen (N) content (NF ISO 10694 and NF ISO 13878, respectively) and
125 texture (NF X 31-107) were then determined (ISO 1995, 1998, 2003). Available calcium (Ca), potassium (K),
126 magnesium (Mg) and phosphorus (P) concentrations were determined using the ammonium acetate-EDTA soil test
127 (Houben et al. 2011).

128

129 **Soil biological properties**

130 Cover crops were destroyed in winter and earthworms and Collembola were sampled in each plot in the next spring
131 (May 2016). Earthworms were collected by chemical extraction with formalin on 0.5 m² quadrats (ISO 23611-1) (ISO
132 2018). Collembola were collected in soil samples obtained by soil corers (diameter 5 cm) that included the organic
133 layer (if present) and the top 5 cm of the organo-mineral horizon, without disturbing the soil profile. In the laboratory,
134 Collembola were extracted during 7 days using the Berlese-Tullgren device (Tullgren 1918). All individuals were
135 preserved in 70% ethanol and identified to the species following different identification keys (Gisin 1960; Bouché
136 1972; Sims and Gerard 1985; Hopkin 1997). Only adults earthworms were identified.

137

138 **Statistical analyses**

139 Shannon-Weaver index ($H = -\sum p_i \ln p_i$, where H is Shannon-Weaver index and p_i is the proportional abundance of
140 species i) was used as a species diversity index of soil fauna (Shannon and Weaver 1949), instead of the Simpson
141 diversity index for its better sensitivity to less abundant species. All recorded data were analyzed using descriptive
142 statistics (mean \pm standard error as $SE = SD/\sqrt{n}$, where SE is standard error, SD is standard deviation and n is the
143 number of replicates) and normality was assessed using the Shapiro-Wilk test. Paired t-tests and paired Wilcoxon tests
144 were used for comparisons of quantitative variables (soil parameters, cover crops characteristics, fauna abundances,
145 species richness and diversity indices) between conventional and CA systems. Univariate statistical analyses were
146 performed on the seven pairs of sites (n=7), averaging the four plots per site, with the R software (R Core Team
147 2013).

148 The null-hypothesis of no difference in assemblage composition between practices was tested using one-way
 149 ANOSIM based on the Bray-Curtis dissimilarity distance configuration (Clarke et al. 2006). SIMPER analysis based
 150 on the Bray-Curtis dissimilarity distance was run to determine the species that contributed most in differentiating the
 151 two groups tested by ANOSIM. These analyses were performed with the free PAST 4.03 software. In order to
 152 visualize the pattern of species composition and explore similarities and dissimilarities between both practices, non-
 153 metric multi-dimensional scaling (NMDS) was used (Anderson 1971), on a data table including the top 9 and top 5
 154 most abundant (>20 ind. per sample) species for earthworms and Collembola, respectively, and the 56 and 37 plots
 155 with at least one individual for earthworms and Collembola, respectively. We used the *vegan* R-package with the
 156 function ‘meta-MDS’ for species ordination by NMDS and the function ‘envfit’ to calculate correlations between
 157 ordination axes and quantitative environmental variables (cover crops characteristics and time since conversion to
 158 CA), where significance is based on 999 random permutations of the data table (Oksanen et al. 2013).
 159

160 Table 1: characteristics of the 14 fields of the study being under four-year rotation. Conv. : conventional, CA:
 161 conservation agriculture system. CC: cover crops. Mech: mechanical, chem: chemical.

Pair	System	CC biomass (g)	CC Simpson index	CC species richness	Time since conversion to CA (years)	Crop	Years between tillage	Depth of tillage (cm)	CC destruction
1	Conv	36	0	1	0	Pea	4	25	Mech
	CA	141	1.54	6.25	5	Pea	0	0	Mech + chem
2	Conv	0	0	0	0	Pea	3	25	0
	CA	247	1.83	7.25	9	Pea	0	0	Mech
3	Conv	0	0	0	0	Corn	3	25	0
	CA	334	1.58	5.5	7	Corn	0	0	Mech
4	Conv	54	1.47	5.5	0	Barley	3	25	Mech + chem
	CA	268	1.55	5.5	2	Barley	0	0	Mech + chem
5	Conv	47	0.67	2	0	Barley	2	20	Mech + chem
	CA	239	1.18	3.75	5	Barley	0	0	Mech
6	Conv	115	0	1	0	Barley	4	25	Mech + chem
	CA	222	1.75	7.5	6	Barley	0	0	Mech
7	Conv	0	0	0	0	Pea	4	20	0
	CA	297	1.54	6.75	6	Pea	0	0	Mech + chem

162
 163
 164

165 **Results**

166

167 **Soil chemical parameters and plant cover characteristics in conventional and conservation agriculture (CA)**
168 **systems**

169 Soil chemical properties did not differ between conventional and CA systems, indicating that pairs of sites presented
170 the same soil characteristics (Table 2). By contrast, plant cover characteristics differed within each pair as CA systems
171 showed on average 7 times higher plant biomass, 4.5 times higher plant species richness, and higher plant diversity than
172 conventional systems (Table 3).

173

174 **Soil fauna in conventional and conservation agriculture (CA) systems**

175 Earthworms total abundance was significantly higher (4.8 times) in CA systems and Collembola total abundance was
176 2.5 times higher in CA systems. The latter difference remains however a trend ($0.05 < p < 0.1$). The same increase pattern
177 was observed for all earthworm species (Table 4) and was significantly different for the three most abundant species
178 (*Lumbricus terrestris*, *Aporrectodea rosea* and *A. caliginosa*) which represented 59% and 65% of the total earthworm
179 abundance in the conventional and the CA system respectively.

180 Only the abundance of the second dominant Collembola species (*Proisotoma minuta*) in conventional system
181 significantly increased in CA system (Table 4). This results in a shift of the dominant Collembola species from the
182 conventional system (*Folsomia brevicauda*, with a share of 28.8% of the community) to the CA system (*P. minuta*
183 making 62.3% of the total abundance; Table 4). Only the most abundant species of Collembola are shown in Table 4,
184 see Table S1 in supplementary data for the whole dataset including rarer species.

185 Fauna species richness and diversity indices did not differ between agricultural systems, even if earthworm species
186 richness tended to be higher in CA systems ($0.05 < p < 0.1$) (Table 4).

187

188 Table 2: soil chemical parameters in two agricultural systems. n=7. Mean values are indicated with (\pm) standard error.
 189 “ns” indicates non significant differences in systems based on paired t-tests or Wilcoxon tests ($\alpha=0.05\%$). CA:
 190 conservation agriculture system.
 191

	Conventional	CA	
Available P (mg kg ⁻¹)	51.4 \pm 13.3	75.6 \pm 24.1	ns
Available K (mg kg ⁻¹)	150.2 \pm 12.0	163.3 \pm 17.4	ns
Available Mg (mg kg ⁻¹)	124.0 \pm 27.1	92.2 \pm 9.8	ns
Available Ca (mg kg ⁻¹)	11398.9 \pm 4559.9	4787.8 \pm 1047.8	ns
Organic C (%)	14.92 \pm 1.91	13.13 \pm 0.87	ns
Total N (%)	0.16 \pm 0.02	0.15 \pm 0.01	ns
C/N	9.50 \pm 0.28	9.10 \pm 0.29	ns
pH	7.63 \pm 0.23	7.67 \pm 0.15	ns
Clay (%)	22.41 \pm 2.03	21.66 \pm 2.54	ns
Silt (%)	65.20 \pm 2.44	64.81 \pm 2.72	ns
Sand (%)	12.38 \pm 1.29	13.53 \pm 1.39	ns

192
 193
 194 Table 3: cover crops (CC) characteristics in two agricultural systems. n=7. Mean values are indicated with (\pm)
 195 standard error. Asterisks indicate significant differences in systems based on paired t-tests or Wilcoxon tests (“***”:
 196 $p<0.001$, “**”: $0.01<p<0.05$). CA: conservation agriculture system.
 197

	Conventional	CA	
CC biomass (g)	36 \pm 16	250 \pm 23	***
CC Simpson index	0.31 \pm 0.22	1.57 \pm 0.08	*
CC species richness	1.36 \pm 0.75	6.07 \pm 0.49	*

198
 199

200 Table 4: soil biological properties in two agricultural systems. n=7, only the top 9 and top 5 most abundant (>20 ind.)
 201 species (for earthworms and Collembola , respectively) are shown, see Table S1 for the whole dataset including rarer
 202 species of Collembola. Mean values are indicated with (\pm) standard error. Asterisks indicate significant differences in
 203 systems in t-tests or Wilcoxon tests (“***”: $p < 0.001$, “**”: $0.01 < p < 0.05$, “.”: $0.05 < p < 0.1$, “ns”: non significant). CA:
 204 conservation agriculture system. Ecological categories are from (Bottinelli et al. 2020) and (Gisin 1943) for
 205 earthworms and Collembola respectively.

206

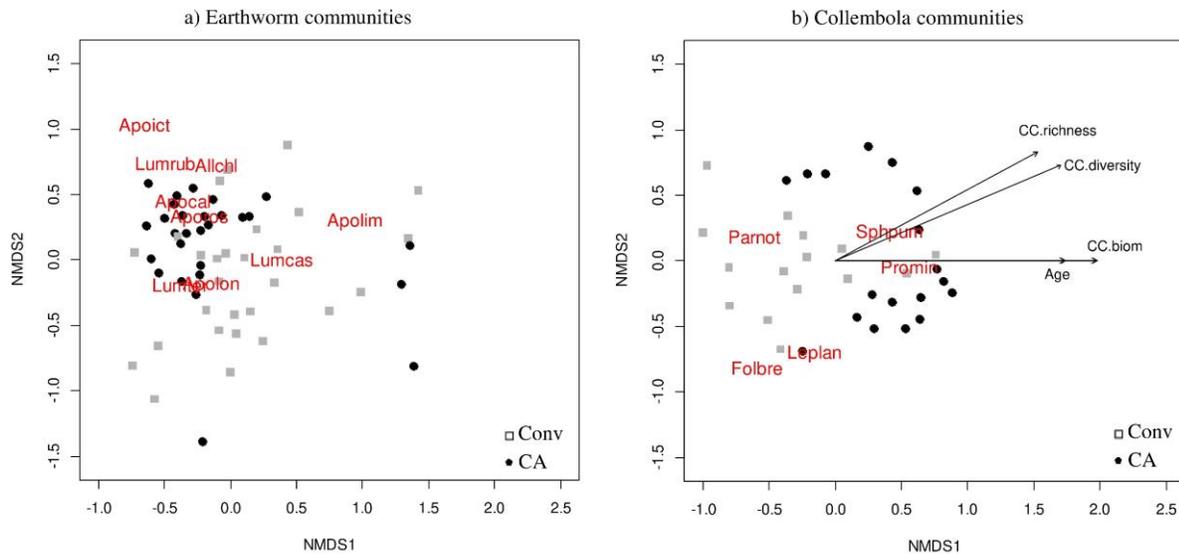
		Ecological categories	Conventional	CA	
Abundance (Ind.m ⁻²)	<i>Aporrectodea longa</i>	Epi-aneic	6.6 \pm 1.8	18.4 \pm 5.7	ns
	<i>Lumbricus terrestris</i>	Epi-aneic	6.1 \pm 2.2	26.4 \pm 6.7	*
	<i>Aporrectodea caliginosa</i>	Endogeic	5.6 \pm 2.2	32.6 \pm 10.0	*
	<i>Aporrectodea rosea</i>	Endogeic	5.5 \pm 1.5	32.4 \pm 14.1	*
	<i>Aporrectodea limicola</i>	unknown	3.1 \pm 1.3	3.6 \pm 1.4	ns
	<i>Lumbricus castaneus</i>	Epigeic	2.1 \pm 0.9	17.2 \pm 12.8	.
	<i>Allolobophora chlorotica</i>	Epi-endo-aneic	0.4 \pm 0.3	4.5 \pm 2.5	ns
	<i>Aporrectodea icterica</i>	Endogeic	0.0 \pm 0.0	3.6 \pm 3.3	ns
	<i>Lumbricus rubellus</i>	Epigeic	0.0 \pm 0.0	2.6 \pm 1.5	ns
Total earthworm abundance (Ind.m ⁻²)			29.4 \pm 4.4	141.4 \pm 26.1	*
Earthworm species richness			3.4 \pm 0.3	5.0 \pm 0.7	.
Earthworm Shannon diversity index			0.36 \pm 0.01	0.37 \pm 0.01	ns
Abundance (Ind.m ⁻²)	<i>Folsomia brevicauda</i>	Hemiedaphic	1248.0 \pm 561.6	2215.2 \pm 2100.8	ns
	<i>Proisotoma minuta</i>	Hemiedaphic	1014.0 \pm 327.6	6879.6 \pm 2095.6	*
	<i>Parisotoma notabilis</i>	Hemiedaphic	769.6 \pm 218.4	369.2 \pm 223.6	ns
	<i>Lepidocyrtus lanuginosus</i>	Epedaphic	655.2 \pm 301.6	400.4 \pm 156.0	ns
	<i>Sphaeridia pumilis</i>	Euedaphic	26.0 \pm 26.0	462.8 \pm 171.6	.
Total Collembola abundance (Ind.m ⁻²)			4331.6 \pm 1014.0	11044.8 \pm 3250.0	.
Collembola species richness			4.3 \pm 0.4	4.1 \pm 0.5	ns
Collembola Shannon diversity index			0.16 \pm 0.02	0.22 \pm 0.02	ns

207

208 Structure of faunal communities in conventional and conservation agriculture systems

209 NMDS ordination of earthworm communities revealed a strong differentiation between the two agricultural systems
 210 (56 plots, 9 species, stress = 0.181, figure 1a), even if 4 plots of CA treatments were separated from all the other CA
 211 plots. The visual differentiation was confirmed by the ANOSIM analysis which showed a significant difference in

212 species composition ($P = 0.0001$, $R=0.31$) and the SIMPER analysis which showed 67.46% of dissimilarity; *A.*
 213 *caliginosa*, *L. terrestris* and *A. rosea* mostly contributing to discriminate conventional and CA systems. In the same
 214 way, NMDS ordination of Collembola communities revealed the same differentiation between systems (37 plots, 5
 215 species, stress = 0.164, figure 1b). The significant difference in species composition (ANOSIM, $P = 0.0001$, $R=0.20$;
 216 SIMPER, 62.96% of dissimilarity) showed that *P. minuta* and *F. brevicauda* mostly contributed to discriminate
 217 between the two agricultural systems.



218
 219
 220 Figure 1: non-metric multidimensional scaling (NMDS) plots based on Bray-Curtis dissimilarity for a) earthworms
 221 (top 9 most abundant species (>20 ind.) in 56 plots) and b) Collembola (top 5 species in 37 plots) in 7 pairs of sites
 222 with contrasted agricultural systems (Conv: conventional system, grey squares; CA: conservation agriculture system,
 223 black dots). Only significant quantitative environmental variables are shown. Time: time since conversion to CA, CC
 224 richness : Cover Crops total species richness, CC diversity : Cover Crops Simpson diversity index, CC biom : Cover
 225 Crops total biomass. Earthworms species: Allchl: *Allolobophora chlorotica*, Apocal: *Aporrectodea caliginosa*,
 226 Apoict: *Aporrectodea icterica*, Apolim: *Aporrectodea limicola*, Apolon : *Aporrectodea longa*, Aporos: *Aporrectodea*
 227 *rosea*, Lumcas: *Lumbricus castaneus*, Lumrub: *Lumbricus rubellus*, Lumter: *Lumbricus terrestris*. Collembola
 228 species: Promin: *Proisotoma minuta*, Folbre: *Folsomia brevicauda*, Parnot: *Parisotoma notabilis*, Sphpum:
 229 *Sphaeridia pumilis*, Leplan: *Lepidocyrtus lanuginosus*.
 230
 231 The ordination of earthworm communities was not influenced by any cover crops variable (biomass, diversity or

232 richness) whereas Collembola communities were significantly influenced by these variables as well as the time since
233 conversion to CA (Table S2), which was highly correlated with cover crops biomass (Figure 1b).

234

235 **Discussion**

236

237 We studied the abundance, diversity and community structure of the soil mesofauna (Collembola) and macrofauna
238 (earthworms) in pairs of plots with contrasting agricultural systems (conventional vs. conservation agriculture (CA))
239 and practices. The two main contrasts between the conventional and CA fields were the use of tillage and the
240 management of cover crops, representing an interesting model to examine the relationship between these practices and
241 the soil fauna communities structure, along with the effect of time since conversion to CA systems on these
242 communities.

243

244 **Conservation agriculture positively influences abundance of soil fauna**

245 Our results supported our first hypothesis (H1) as CA systems promoted the abundance of earthworm and Collembola
246 communities. A clear positive effect of CA systems was indeed observed on the total abundance of earthworms, as
247 well as on the abundance of the most abundant earthworm species, which is in line with previous studies (Henneron
248 et al. 2015; Briones and Schmidt 2017). It would have been preferable to supplement formalin earthworms extraction
249 with a sampling by digging and handsorting since some species may be more affected than others by formalin
250 extraction (Smith et al. 2008; Lagerlöf et al. 2012). However, as the extraction method was the same in both
251 modalities studied (conventional vs. CA), we assume that the comparison between these modalities remains relevant.
252 Collembola abundance tended to respond positively to conservation agriculture. As larger organisms, earthworms
253 appear to be generally more sensitive to tillage operations than smaller organisms such as Collembola, due to the
254 physical disruption of the soil, the incorporation of crop residue and the resulting changes in soil water and
255 temperature (Wardle 1995; Kladravko 2001). CA systems in this study were also characterized by a higher cover crop
256 residue biomass which, by providing more food to soil fauna communities, may also contribute to increase their
257 population (Gatiboni et al. 2011; Sauvadet et al. 2016). The lack of significant difference between most of Collembola
258 species abundance in CA and conventional systems could be associated with the high diversity of agricultural
259 practices included in the “conservation agriculture” term, especially the management of cover crops and its

260 consequence for residues quantity and quality (Hobbs 2007). This was partly confirmed by the NMDS graphical
261 representation with 4 CA plots clearly differentiated from the other CA plots. On the other hand, this higher
262 variability of fauna abundance may be due to a higher variability in the response of faunal groups, especially
263 Collembola, which may be positively or negatively impacted by CA systems due to the contrasted effects of crop
264 residue on Collembola abundance already noticed in the litterature (Brennan et al. 2006; Scheunemann et al. 2015;
265 Sauvadet et al. 2016). Indeed, before benefitting Collembola, cover crops residues may require time to induce higher
266 abundance of saprotrophic microorganisms (Scheunemann et al. 2015). Furthermore, while providing a favorable
267 shelter for Collembola against microclimatic fluctuations, cover crop residues may also favour the presence of their
268 predators, thereby increasing top-down regulation (Brennan et al. 2006). Therefore, earthworms and Collembola
269 communities constitute relevant indicators of sustainable cropping systems, as they positively respond to CA systems,
270 in particular Collembola to cover crops management (biomass productivity, species diversity...).

271 Unlike abundance, earthworm and Collembola species richness and diversity was not improved by CA systems, which
272 partially contradicts our first hypothesis and, to a lesser extent, the general idea that CA promotes soil fauna diversity
273 notably due to higher aboveground plant diversity leading to higher quality litter (van Capelle et al. 2012; Sauvadet et
274 al. 2016; Coulibaly et al. 2017). It has been demonstrated that plant diversity stabilizes the belowground diversity of
275 microbial and faunal species (Milcu et al. 2010), even if addition of crop residues may have contrasted effects on
276 Collembola species diversity (Brennan et al. 2006; Scheunemann et al. 2015). After a conversion from conventional to
277 CA systems, it may take several years for new species to recolonize the site (Coulibaly et al. 2017), especially within
278 a vast agricultural area probably impoverished in soil fauna (Tsiafouli et al. 2015).

279

280 **Effect of cover crops management, tillage and time since conversion to CA on soil fauna communities structure**

281 As assumed in our second hypothesis, both earthworms and Collembola communities presented a clear differentiation
282 in agricultural practices. The strong effect of the practice on the abundance of the predominant species of earthworms
283 (*L. terrestris*, *A. rosea* and *A. caliginosa*) and Collembola (*P. minuta*) was responsible for differentiation of the
284 communities. Species living in the intermediate soil layers (as *L. terrestris*, *P. minuta* and *F. brevicauda* in this study)
285 are generally promoted by CA systems (van Capelle et al. 2012; Henneron et al. 2015; Briones and Schmidt 2017;
286 Coulibaly et al. 2017), as the reduction of physical disturbances in the intermediate soil layers reduces the stress and
287 environmental filter on those species and allows their population to grow over time (Coulibaly et al. 2017).

288 Earthworms could be more affected by tillage than by the presence of a cover crop (Kladivko 2001), but Collembola,
289 being mainly bottom-up regulated, may also benefit from a more stable resource supply (fungal mycelium) in those
290 systems with a lower mechanical disturbance (Coulibaly et al. 2017).

291
292 Our results highlighted that the enhancement of Collembola by CA systems may be accentuated by the time since
293 conversion to CA (*i.e.* the number of years since the conversion of conventional system to CA system), possibly due
294 to a better management of cover crops by the more experienced farmers (as evidenced by the high correlation between
295 the time since conversion to CA and cover crop biomass). Although this was previously reported for soil
296 microorganisms (Houben et al. 2018) these findings constitute an important new insight for Collembola which has
297 been rarely highlighted (Coulibaly et al. 2017). Temporal continuity of the habitat would also play a positive role on
298 diversity of Collembola community and could constitute an important parameter to consider in order to manage soil
299 fauna and their associated services (Heiniger et al. 2014). Our study may not have highlighted any effect of the time
300 since conversion to CA on earthworm community because the system change was introduced too recently. Indeed,
301 earthworm population sizes tended to show a greater response to reduced tillage over time, with a greater effect of
302 practices in place for at least 10-20 years compared to studies with cultivation duration of less than 10 years (Briones
303 and Schmidt 2017).

304
305 Cover crops variables (biomass, Simpson index and species richness) did not seem to influence earthworms
306 community structure, while they had a strong significant effect on Collembola community structure. This supports the
307 idea of a strong relationship between plants and Collembola as found in different ecosystems: in forests and
308 grasslands both, several studies reported an important link between vegetation and soil Collembola (Sabais et al.
309 2011; Henneron et al. 2017; Forey et al. 2018). Different hypotheses may explain this link but our results (*i.e.* the
310 strong positive effect of cover crops diversity) support the mass-ratio hypothesis suggesting that plant community
311 identity and composition would drive Collembola assemblages by controlling dominant plants traits (*e.g.* Wardle
312 1995) or perhaps more precisely by controlling plant residuals traits after harvest.

313

314 **Conclusion**

315 Our study shows rather positive effects of alternative agricultural practices such as CA on soil mesofauna

316 (Collembola), and an even more visible effect on macrofauna (earthworms), maybe due to less physical disturbance of
 317 the soil (no-tillage), and the presence of cover crops that favours soil organism abundance by serving as shelter and
 318 supplying food when destroyed. The composition of these communities is clearly structured by agricultural practices.
 319 In particular, species living or burrowing at intermediate soil depth were predominantly promoted under CA systems
 320 and could therefore be used as indicators for agri-environmental management at the cropping system level. However,
 321 cover crop management in conservation agriculture may be dependent on the use of herbicides, and their detrimental
 322 effect on soil fauna communities needs to be more deeply investigated (Renaud et al. 2004; García-Pérez et al. 2014).
 323 Further research should also be carried out to understand the relationships between cover crops management and
 324 fauna assemblages by examining functional traits and functional diversity of above- and belowground crop residues
 325 and fauna communities.

326

327 *Table S1: abundance of the rare Collembola species. Mean values are indicated with (\pm) standard error. CA:*
 328 *conservation agriculture system. Ecological categories are from (Gisin 1943).*

		Ecological categories	Conventional	CA
Total abundance	<i>Lepidocyrtus cyaneus</i>	Hemiedaphic	0.31 \pm 0.24	0.11 \pm 0.07
	<i>Pseudosinella alba</i>	Hemiedaphic	0.36 \pm 0.28	0.07 \pm 0.07
	<i>Sminthurus viridis</i>	Euedaphic	0.05 \pm 0.05	0.32 \pm 0.25
	<i>Isotomurus antennalis</i>	Euedaphic	0.19 \pm 0.19	0.52 \pm 0.22
	<i>Stenaphorura quadrispina</i>	Epedaphic	0.38 \pm 0.38	0.17 \pm 0.11
	<i>Isotomiella minor</i>	Hemiedaphic	0.26 \pm 0.14	0.25 \pm 0.21

329

330 *Table S2: correlation values between earthworm and Collembola NMDS axes and environmental variables*
 331 *(agricultural practices). CA: conservation agriculture system. Asterisks indicate significant correlations (“***”:*
 332 *$p < 0.001$, “. ”: $0.05 < p < 0.1$, “ns”: non significant).*

	Correlation with earthworm NMDS axes	Correlation with Collembola NMDS axes
Cover crops biomass (g)	ns	***
Cover crops Simpson index	ns	***
Cover crops species richness	.	***
Time since conversion to CA	ns	***

333

334

335 **References**

336

337 Anderson AJB (1971) Ordination Methods in Ecology. *Journal of Ecology* 59:713–726.
338 <https://doi.org/10.2307/2258135>

339 Bardgett RD, van der Putten WH (2014) Belowground biodiversity and ecosystem functioning. *Nature* 515:505–511.
340 <https://doi.org/10.1038/nature13855>

341 Bender SF, van der Heijden MGA (2015) Soil biota enhance agricultural sustainability by improving crop yield,
342 nutrient uptake and reducing nitrogen leaching losses. *Journal of Applied Ecology* 52:228–239.
343 <https://doi.org/10.1111/1365-2664.12351>

344 Blouin M, Hodson ME, Delgado EA, et al (2013) A review of earthworm impact on soil function and ecosystem
345 services. *European Journal of Soil Science* 64:161–182. <https://doi.org/10.1111/ejss.12025>

346 Bottinelli N, Hedde M, Jouquet P, Capowiez Y (2020) An explicit definition of earthworm ecological categories –
347 Marcel Bouché’s triangle revisited. *Geoderma* 372:114361.
348 <https://doi.org/10.1016/j.geoderma.2020.114361>

349 Bouché MB (1972) *Lombriciens de France. Ecologie et systématique*. INRA Editions

350 Brennan A, Fortune T, Bolger T (2006) Collembola abundances and assemblage structures in conventionally tilled
351 and conservation tillage arable systems. *Pedobiologia* 50:135–145.
352 <https://doi.org/10.1016/j.pedobi.2005.09.004>

353 Briones MJI, Schmidt O (2017) Conventional tillage decreases the abundance and biomass of earthworms and alters
354 their community structure in a global meta-analysis. *Global Change Biology* 23:4396–4419.
355 <https://doi.org/10.1111/gcb.13744>

356 Chan KY (2001) An overview of some tillage impacts on earthworm population abundance and diversity —
357 implications for functioning in soils. *Soil and Tillage Research* 57:179–191. [https://doi.org/10.1016/S0167-](https://doi.org/10.1016/S0167-1987(00)00173-2)
358 [1987\(00\)00173-2](https://doi.org/10.1016/S0167-1987(00)00173-2)

359 Clarke KR, Somerfield PJ, Chapman MG (2006) On resemblance measures for ecological studies, including
360 taxonomic dissimilarities and a zero-adjusted Bray–Curtis coefficient for denuded assemblages. *Journal of*
361 *Experimental Marine Biology and Ecology* 330:55–80. <https://doi.org/10.1016/j.jembe.2005.12.017>

362 Corbeels M, Scopel E, Cardoso A, et al (2006) Soil carbon storage potential of direct seeding mulch-based cropping
363 systems in the Cerrados of Brazil: SOIL C STORAGE UNDER DMC IN THE CERRADOS. *Global*
364 *Change Biology* 12:1773–1787. <https://doi.org/10.1111/j.1365-2486.2006.01233.x>

365 Coudrain V, Hedde M, Chauvat M, et al (2016) Temporal differentiation of soil communities in response to arable
366 crop management strategies. *Agriculture, Ecosystems & Environment* 225:12–21.
367 <https://doi.org/10.1016/j.agee.2016.03.029>

368 Coulibaly SFM, Aubert M, Brunet N, et al (2022) Short-term dynamic responses of soil properties and soil fauna
369 under contrasting tillage systems. *Soil and Tillage Research* 215:105191.
370 <https://doi.org/10.1016/j.still.2021.105191>

371 Coulibaly SFM, Coudrain V, Hedde M, et al (2017) Effect of different crop management practices on soil
372 Collembola assemblages: A 4-year follow-up. *Applied Soil Ecology* 119:354–366.
373 <https://doi.org/10.1016/j.apsoil.2017.06.013>

- 374 Coulibaly SFM, Winck BR, Akpa-Vinceslas M, et al (2019) Functional Assemblages of Collembola Determine Soil
375 Microbial Communities and Associated Functions. *Front Environ Sci* 7:52.
376 <https://doi.org/10.3389/fenvs.2019.00052>
- 377 Culik MP, de Souza JL, Ventura JA (2002) Biodiversity of Collembola in tropical agricultural environments of
378 Espírito Santo, Brazil. *Applied Soil Ecology* 21:49–58. [https://doi.org/10.1016/S0929-1393\(02\)00048-3](https://doi.org/10.1016/S0929-1393(02)00048-3)
- 379 Denier J, Faucon M-P, Dulaurent A-M, et al (2022) Earthworm communities and microbial metabolic activity and
380 diversity under conventional, feed and biogas cropping systems as affected by tillage practices. *Applied Soil*
381 *Ecology* 169:104232. <https://doi.org/10.1016/j.apsoil.2021.104232>
- 382 Eriksen-Hamel NS, Speratti AB, Whalen JK, et al (2009) Earthworm populations and growth rates related to long-
383 term crop residue and tillage management. *Soil and Tillage Research* 104:311–316.
384 <https://doi.org/10.1016/j.still.2009.04.006>
- 385 FAO (2022) Conservation Agriculture. <http://www.fao.org/conservation-agriculture/en/>. Accessed 19 Jul 2022
- 386 Filser J (2002) The role of Collembola in carbon and nitrogen cycling in soil: Proceedings of the Xth international
387 Colloquium on Apterygota, České Budějovice 2000: Apterygota at the Beginning of the Third Millennium.
388 *Pedobiologia* 46:234–245. <https://doi.org/10.1078/0031-4056-00130>
- 389 Forey E, Chauvat M, Coulibaly SFM, et al (2018) Inoculation of an ecosystem engineer (Earthworm: *Lumbricus*
390 *terrestris*) during experimental grassland restoration: Consequences for above and belowground soil
391 compartments. *Applied Soil Ecology* 125:148–155. <https://doi.org/10.1016/j.apsoil.2017.12.021>
- 392 Frazão J, de Goede RGM, Salánki TE, et al (2019) Responses of earthworm communities to crop residue
393 management after inoculation of the earthworm *Lumbricus terrestris* (Linnaeus, 1758). *Applied Soil*
394 *Ecology* 142:177–188. <https://doi.org/10.1016/j.apsoil.2019.04.022>
- 395 García-Pérez JA, Alarcón-Gutiérrez E, Perroni Y, Barois I (2014) Earthworm communities and soil properties in
396 shaded coffee plantations with and without application of glyphosate. *Applied Soil Ecology* 83:230–237.
397 <https://doi.org/10.1016/j.apsoil.2013.09.006>
- 398 Gatiboni LC, Coimbra JLM, Denardin RBN, Prado Wildner L do (2011) Microbial biomass and soil fauna during the
399 decomposition of cover crops in no-tillage system. *Rev Bras Ciênc Solo* 35:1051–1057.
400 <https://doi.org/10.1590/S0100-06832011000400008>
- 401 Ghosh S, Das TK, Sharma DK, Gupta K (2019) Potential of conservation agriculture for ecosystem services: A
402 review. *Indian Journal of Agricultural Sciences* 89:1572–9
- 403 Gisin H (1960) *Collembolenfauna Europas*, Museum d'histoire naturelle. Geneva
- 404 Gisin H (1943) *Okologie und Lebensgemeinschaften der Collembolen im schweizerischen Exkursionsgebiet Basels*.
405 *Revue Suisse de Zoologie* 50:131–224
- 406 Heiniger C, Barot S, Ponge J-F, et al (2014) Effect of habitat spatiotemporal structure on collembolan diversity.
407 *Pedobiologia* 57:103–117. <https://doi.org/10.1016/j.pedobi.2014.01.006>
- 408 Henneron L, Aubert M, Archaux F, et al (2017) Forest plant community as a driver of soil biodiversity: experimental
409 evidence from collembolan assemblages through large-scale and long-term removal of oak canopy trees
410 *Quercus petraea*. *Oikos* 126:420–434. <https://doi.org/10.1111/oik.03677>
- 411 Henneron L, Bernard L, Hedde M, et al (2015) Fourteen years of evidence for positive effects of conservation
412 agriculture and organic farming on soil life. *Agron Sustain Dev* 35:169–181.
413 <https://doi.org/10.1007/s13593-014-0215-8>

414 Hobbs PR (2007) Conservation agriculture: what is it and why is it important for future sustainable food production?
415 *The Journal of Agricultural Science* 145:127–137. <https://doi.org/10.1017/S0021859607006892>

416 Holland JM (2004) The environmental consequences of adopting conservation tillage in Europe: reviewing the
417 evidence. *Agriculture, Ecosystems & Environment* 103:1–25. <https://doi.org/10.1016/j.agee.2003.12.018>

418 Hopkin SP (1997) *Biology of the Springtails (Insecta: Collembola)*. Oxford University Press

419 Houben D, Faucon M-P, Mercadal A-M (2018) Response of Organic Matter Decomposition to No-Tillage Adoption
420 Evaluated by the Tea Bag Technique. *Soil Systems* 2:42. <https://doi.org/10.3390/soilsystems2030042>

421 Houben D, Meunier C, Pereira B, Sonnet Ph (2011) Predicting the degree of phosphorus saturation using the
422 ammonium acetate–EDTA soil test. *Soil Use and Management* 27:283–293. [https://doi.org/10.1111/j.1475-](https://doi.org/10.1111/j.1475-2743.2011.00353.x)
423 [2743.2011.00353.x](https://doi.org/10.1111/j.1475-2743.2011.00353.x)

424 ISO (1995) NF ISO 10694. Qualité du sol – Dosage du carbone organique et du carbone total après combustion
425 sèche.

426 ISO (1998) NF ISO 13878. Qualité du sol – Détermination de la teneur totale en azote par combustion sèche
427 (“analyse élémentaire”)

428 ISO (2003) NF X 31-107. Qualité du sol – Détermination de la distribution granulométrique des particules du sol –
429 Méthode à la pipette

430 ISO (2018) ISO 23611 Soil quality — Sampling of soil invertebrates — Part 1: Hand-sorting and extraction of
431 earthworms.

432 Kladivko EJ (2001) Tillage systems and soil ecology. *Soil and Tillage Research* 61:61–76.
433 [https://doi.org/10.1016/S0167-1987\(01\)00179-9](https://doi.org/10.1016/S0167-1987(01)00179-9)

434 Lagerlöf J, Pålsson O, Arvidsson J (2012) Earthworms influenced by reduced tillage, conventional tillage and energy
435 forest in Swedish agricultural field experiments. *Acta Agriculturae Scandinavica, Section B — Soil & Plant*
436 *Science* 62:235–244. <https://doi.org/10.1080/09064710.2011.602717>

437 Météo France (2016) Public data from Météo-France. <https://donneespubliques.meteofrance.fr/>. Accessed 25 Oct
438 2022

439 Milcu A, Thebault E, Scheu S, Eisenhauer N (2010) Plant diversity enhances the reliability of belowground
440 processes. *Soil Biology and Biochemistry* 42:2102–2110. <https://doi.org/10.1016/j.soilbio.2010.08.005>

441 Oksanen J, Kindt R, O’Hara B (2013) *Community Ecology Package*. R package version 20

442 Pelosi C, Pey B, Hedde M, et al (2014) Reducing tillage in cultivated fields increases earthworm functional diversity.
443 *Applied Soil Ecology* 83:79–87. <https://doi.org/10.1016/j.apsoil.2013.10.005>

444 Ponge JF, Gillet S, Dubs F, et al (2003) Collembolan communities as bioindicators of land use intensification. *Soil*
445 *Biology and Biochemistry* 35:813–826. [https://doi.org/10.1016/S0038-0717\(03\)00108-1](https://doi.org/10.1016/S0038-0717(03)00108-1)

446 Ponge J-F, Pérès G, Guernion M, et al (2013) The impact of agricultural practices on soil biota: A regional study.
447 *Soil Biology and Biochemistry* 67:271–284. <https://doi.org/10.1016/j.soilbio.2013.08.026>

448 Postma-Blaauw MB, de Goede RGM, Bloem J, et al (2010) Soil biota community structure and abundance under
449 agricultural intensification and extensification. *Ecology* 91:460–473. <https://doi.org/10.1890/09-0666.1>

450 R Core Team (2013) *R: A language and environment for statistical computing*

- 451 Renaud A, Poinot-Balaguer N, Cortet J, Le Petit J (2004) Influence of four soil maintenance practices on
452 Collembola communities in a Mediterranean vineyard. *Pedobiologia* 48:623–630.
453 <https://doi.org/10.1016/j.pedobi.2004.07.002>
- 454 Sabais ACW, Scheu S, Eisenhauer N (2011) Plant species richness drives the density and diversity of Collembola in
455 temperate grassland. *Acta Oecologica* 37:195–202. <https://doi.org/10.1016/j.actao.2011.02.002>
- 456 Sauvadet M, Chauvat M, Cluzeau D, et al (2016) The dynamics of soil micro-food web structure and functions vary
457 according to litter quality. *Soil Biology and Biochemistry* 95:262–274.
458 <https://doi.org/10.1016/j.soilbio.2016.01.003>
- 459 Scheunemann N, Maraun M, Scheu S, Butenschoen O (2015) The role of shoot residues vs. crop species for soil
460 arthropod diversity and abundance of arable systems. *Soil Biology and Biochemistry* 81:81–88.
461 <https://doi.org/10.1016/j.soilbio.2014.11.006>
- 462 Shannon C, Weaver W (1949) The mathematical theory of communication. In: *The Mathematical Theory of*
463 *Communication*, Univ. of Illinois Press. Urbana
- 464 Simpson EH (1949) Measurement of Diversity. *Nature* 163:688–688. <https://doi.org/10.1038/163688a0>
- 465 Sims RW, Gerard BM (1985) *Earthworms: Keys and Notes for the Identification and Study of the Species*. Brill
466 Archive
- 467 Smith RG, McSwiney CP, Grandy AS, et al (2008) Diversity and abundance of earthworms across an agricultural
468 land-use intensity gradient. *Soil and Tillage Research* 100:83–88. <https://doi.org/10.1016/j.still.2008.04.009>
- 469 Tsiafouli MA, Thébault E, Sgardelis SP, et al (2015) Intensive agriculture reduces soil biodiversity across Europe.
470 *Global Change Biology* 21:973–985. <https://doi.org/10.1111/gcb.12752>
- 471 Tullgren A (1918) Ein sehr einfacher Ausleseapparat für terricole Tierformen. *Zeitschrift Für Angewante*.
472 *Entomologie* 4:149–150
- 473 van Capelle C, Schrader S, Brunotte J (2012) Tillage-induced changes in the functional diversity of soil biota – A
474 review with a focus on German data. *European Journal of Soil Biology* 50:165–181.
475 <https://doi.org/10.1016/j.ejsobi.2012.02.005>
- 476 Wardle DA (1995) Impacts of Disturbance on Detritus Food Webs in Agro-Ecosystems of Contrasting Tillage and
477 Weed Management Practices. In: Begon M, Fitter AH (eds) *Advances in Ecological Research*. Academic
478 Press, pp 105–185

479

480 **Statements and Declarations**

481 *Funding* The project was partly funded by the Association pour la Promotion d'une Agriculture Durable (APAD).

482 *Competing interests* The authors have no competing interests.

483 *Authors' contributions* Anne-Maïmiti Dulaurent carried out the experiment and performed the analyses with support
484 from Nicolas Honvault and wrote the manuscript with support from David Houben, Michel-Pierre Faucon and
485 Matthieu Chauvat.

486 *Data availability* The datasets generated during and/or analysed during the current study are available from the

487 corresponding author on reasonable request.