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Version of Record: https://www.sciencedirect.com/science/article/pii/S0929139321000196
Manuscript_19a6f76f9b56a7af50279510cc816631

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Abstract:

Fusarium graminearum is a plant-pathogenic fungus that causes the devastating disease "Fusarium head blight" (FHB) in cereal crops such as wheat (*Triticum aestivum*). It also contaminates grains with mycotoxins, including deoxynivalenol (DON), which turn toxic to humans and animals. This fungus overwinters in crop residues left in the field. The fate of mycotoxins in these crop residues in the soil and their ecological role are still unexplored. Therefore, our objective was to assess whether mycotoxins are maintained in the soil, impact the soil biome and benefit the survival of *F. graminearum.* A six-month study in microcosms was performed to examine the fate of DON in *F. graminearum*-contaminated wheat straw and soil, and its impact on soil communities. DON was extracted from straw and soil mixtures, and quantified by high-performance liquid chromatography (HPLC). *Fusarium graminearum* and total fungal and bacterial molecular biomasses were quantified using real-time polymerase chain reaction (Q-PCR). Nematode and earthworm densities were quantified through binocular observations. Changes in the genetic structure of fungi, bacteria, protozoa, and nematodes communities were determined by terminal restriction fragment length polymorphism (T-RFLP) analyses. Results revealed that DON disappeared from the straw and the soil over time. The rate of disappearance was accelerated when straw was incorporated into the soil and when microcosms contained earthworms. Fungal and bacterial biomass, first stimulated during the incorporation of straw, decreased after 2 weeks and until the end of the experiment (24 weeks). It decreased more strongly in the presence of DON. This negative impact of DON was temporary and at the end of the experiment, the bacterial and fungal biomass was higher in the treatments that received DON than in the other treatments while the population of *F. graminearum* was unaffected. Similarly, DON modified the community structures of fungi, bacteria and protozoa to various extents but not that of nematodes. DON-

contaminated straw was found attractive for earthworms, and its presence stimulated their reproduction or cocoon hatching. The major conclusion is that DON briefly affected soil communities, disappeared over time and gave no observable advantage to soil-borne *F. graminearum* populations.

Keywords: mycotoxins, deoxynivalenol, soil-borne *Fusarium graminearum*, wheat straw, soil, microflora, soil fauna, community structure

1. Introduction

Mycotoxins are fungal secondary metabolites potentially harmful to humans and animals. Trichothecenes are among the most important mycotoxins produced in the field by the genus *Fusarium* and are considered as a great threat to humans and animals (Rocha et al., 2005; Tamburic-Ilincic et al., 2015; Mishra et al., 2016; Bilska et al., 2018). They are produced in the field during crop invasion by the pathogen. Their incidence in cereal crops is a huge challenge for the agricultural industry (Tanaka et al., 1988; Bottalico and Perrone, 2002; Oldenburg et al., 2017). Deoxynivalenol (DON) is one of the main trichothecene metabolites; it is found in cereal crops such as wheat (*Triticum aestivum*), rye (*Secale cereale* L.), barley (*Hordeum vulgare*), and maize (*Zea mays*) (Nielsen et al., 2012; Oldenburg et al., 2017; Janssen et al., 2018). DON (also known as vomitoxin) is a type-B trichothecene and is mainly produced by *F. graminearum* (Megalla et al 1986; Tanaka et al 1988). It is a commonly reported mycotoxin associated with Fusarium head blight (FHB) diseased cereals (Paul et al., 2005; Foroud and Eudes, 2009; De Almeida et al., 2016;). DON has very destructive effects on mammals, e.g. immunity reduction, protein biosynthesis damage, food refusal, diarrhea, vomiting, and other severe disease symptoms (Placinta et al., 1999; Fokunang et al., 2006;

Wild and Gong, 2010; Payros et al., 2016). The European Union has set the threshold level for DON in winter wheat to 1.250 mg kg^{-1} (CE N°1116/2007). Beyer et al. (2007) reported that 4.3 % of Fusarium-damaged kernels reach or exceed this limit.

FHB is spread worldwide, and different approaches have been investigated to overcome the disease such as disease forecasting, cultivation of resistant varieties, use of fungicides, agricultural practices, and biocontrol agents (Schisler et al., 2002; De Wolf et al., 2003; Buerstmayr et al., 2009; Prandini et al., 2009; Mesterhazy, 2014; Lemmens et al., 2016; Sarrocco et al., 2019; Rojas et al., 2020). Despite all these experimental investigations, proposing a durable solution for FHB control is still a challenge for agriculture round the world (Venkatesh and Keller, 2019). Disease management is an ultimate tool to reduce related mycotoxins and food losses to make food secure for the increasing world population (Wang et al., 2019). Some mycotoxins like DON are reported to play an active role in fungal aggressiveness. They are not indispensable for initial infection by the fungus, but they favour the spread of FHB within a spike (Bai et al., 2002; Mesterházy, 2002; Mudge et al., 2006).

Crop residues are important components of arable soil functioning thanks to the nutrients they bring back to the soil. They stimulate a high diversity of decomposers in the process of litter decomposition and fuel multitrophic interactions among various soil inhabitants with noticeable consequences on biogeochemical cycles (Perez et al., 2008; Turmel et al., 2015). Potential mechanisms include fungus-driven nutrient transfer among litter species, inhibition or stimulation of microorganisms by specific litter compounds, and a positive feedback of the soil fauna due to greater habitat and food diversity (Rantalainen et al., 2004; Hättenschwiler et al., 2005; Sauvadet et al., 2016). The way the species richness of decomposer fungi, bacteria, as well as the soil fauna including protozoa, or their relative frequencies of occurrence (i.e. community structure) influence the decomposition of organic matter in arable soil is poorly known as compared to forest litter soil (Buee et al., 2009; Barbi et al 2014). Using different

74 sets of fungi isolated from plant litters, Deacon et al., (2006) showed a high degree of
75 functional redundancy in assemblages of culturable decomposer fungi that could buffer the
76 impact of external events on the decomposition process. Therefore, one may wonder how
77 phytopathogenic fungi can be included in this food web during their saprotrophic phase and
78 survive the dynamics of successions. One example of a very strong and effective interaction is
79 provided by the common earthworms (*Lumbricus terrestris* L), which remove sources of
80 phytopathogenic fungi (*Venturia inaequalis*) in orchards by grazing on the leaf litter (Holb et
81 al., 2006).
82 Diseased crop residues (grains, straw and stubble) are colonised by the fungus and are the
83 source of saprophytic survival during off seasons (Pereyra and Dill-Macky, 2008; Sarrocco et
84 al., 2012; Leplat et al., 2016;). Current agriculture is moving towards reducing soil tillage
85 practices to conserve the field soil structure and biotic activity (Bai and Shaner, 2004).
86 However, this reduced tillage favours the survival of the fungus more than deep tillage (Sipila
87 et al., 2012; Hofgaard et al., 2016). *Fusarium graminearum* survives as a saprotroph in crop
88 residues and serves as a primary inoculum to the next crop when warm and moist climatic
89 conditions lead to perithecium formation and ascospore dispersal (Parry et al., 2007; Trail,
90 2009).
91 Crop residues appear as a very coveted resource, but *F. graminearum* is not such a good
92 saprophytic competitor (Leplat et al., 2013) unless the mycotoxins it produces *in planta*
93 provide it with a significant competitive advantage over the microbial and soil fauna
94 communities. Few studies focus on the presence of mycotoxins in the soil and how they
95 interact with the soil biota (Sarrocco et al., 2012; Venkatesh and Keller, 2019). *Lumbricus*
96 *terrestris* earthworm has been found to prefer DON contaminated wheat straw over
97 uncontaminated straw, which resulted in a decrease of the infectious potential associated with
98 the presence of the pathogen, as well as a decrease in DON amounts (Wolfarth et al., 2011).

Other fauna components such as nematodes and collembolans also contribute to soil DON regulation (Wolfarth et al., 2016). The fate of mycotoxins in crop residues and in the soil remains to be addressed to understand the role of DON in the ecological habitat of microorganisms.

Based on this background, the main objective of this study is to understand the ecological role of DON during *F. graminearum* survival in crop residues by monitoring the survival of other soil biota including fungi, bacteria, protozoa, nematodes, and earthworms. Therefore, this study aims at testing the following hypotheses: (1) DON gives a competitive advantage to *F. graminearum* over fungal microflora; (2) DON increases the palatability of straw for earthworms and components of soil fauna; (3) the presence of DON stimulates soil microflora-microfauna interactions

2. Materials and methods

2.1- Soil and straw collection

The soil was collected in May 2010 from the Apk horizon of a Calcaric Cambisol (FAO World Reference Base Soil Classification system, WRB, 2014) on which a permanent meadow grows near a greenhouse area (Latitude: 47°19'1.349"; Longitude: 5°4'25.692") at the INRAE (National Research Institute for Agriculture, Food and Environment, Dijon, France) site. This soil was preferred to cultivated soil to avoid a recent history with wheat and DON. Briefly, the physico-chemical characteristics of this silty clay soil are as follows: 39.2 % clay, 45.6 % silt and 15.2 % sand, 2.05 % organic matter, pH 8, C/N ratio 9.85. The surface of the soil was stripped over 2 cm to remove the grass and part of their root systems. The soil was then taken with a spade, to a depth of 15 cm and placed on metal trays on which it was crumbled by hand. The trays were placed in an oven at 17 ° C for 48 hours to partially dry the soil and facilitate sieving. It was sieved to 4 mm to remove gravels and crop debris. A

123 homogenous mixture of the soil was prepared by manual mixing. The soil was then stored in
124 an incubator at 17°C for 2 weeks. When using it to prepare microcosms, the soil moisture was
125 adjusted to 17 % w/w, *i.e*. 80 % of its water-holding capacity.
126 Air-dry straw was taken from winter wheat (*Triticum aestivum*) originating from a field of the
127 INRAE Experimental Unit (Bretenières, France). The straw was shredded into approximately
128 2-3 cm pieces.

129 **2.2- *Fusarium graminearum* inoculum**

130 A previously described DON-chemotype *F. graminearum* strain MIAE00376 (Leplat et al.,
131 2016) was provided by the collection "Microorganisms of Interest for Agriculture and
132 Environment" (MIAE, Agroécologie, INRAE Dijon, France).
133 Conidia were produced by the method adapted from Hassan and Bullerman (2009). Briefly,
134 wheat bran liquid medium was prepared by adding 1% of wheat bran in distilled water and
135 was autoclaved at 120°C for 20 min. Small plugs of 7-day-old cultures of *F. graminearum*
136 strain MIAE00376 grown on potato dextrose agar (PDA) were added, and flasks were placed
137 at 25°C on a rotary shaker (150 rpm) for seven days. The cultures were filtered through sterile
138 cheesecloth (50 µm mesh size) to remove the mycelium mat and remaining pieces of bran.
139 The conidial concentration was assessed under the microscope using a Malassez counting
140 chamber. The concentration (6.7×10^2 conidia ml^{-1}) was then adjusted with sterile distilled
141 water to achieve a final concentration of 10^3 conidia g^{-1} straw (see below).

142 **2.3- Collection of earthworms**

143 Adult *L. terrestris* specimens were caught from the permanent meadow near the greenhouse
144 area where soil was collected, at the INRAE site in May 2010. The soil was thoroughly
145 watered 2 days before catching earthworms, and then a new moderate watering using 0.5 %
146 formalin in water was performed to compel earthworms to come out from the depth to the soil
147 surface (Bouché, 1972). The collected adult *L. terrestris* were washed immediately twice

148 using tap water to remove the formalin from their skins. They were added to the soil of
149 different containers and placed at 10°C for 3 weeks. At the start of the experiment,
150 earthworms were added to the experimental soil at 17°C for 4 days.

151 **2.4- Experimental design**

152 The study was performed in microcosms with soil- and *F. graminearum*-infected straw
153 contaminated or non-contaminated with DON. The experiment had a completely randomized
154 block design with three replications, five sampling times and including the following
155 treatments:

156 1. SS: Straw at the Surface
157 2. SSD: Straw at the Surface + DON
158 3. SSE: Straw at the Surface + Earthworms
159 4. SSDE: Straw at the Surface + DON + Earthworms
160 5. SME: Straw incorporated to the soil + Earthworms
161 6. SMDE: Straw incorporated to the soil + DON + Earthworms

162 These microcosms were placed in an incubator at 17°C. Their moisture was measured
163 regularly by weighing them and was adjusted by spraying water when needed. The sample of
164 the representative homogenous DON-contaminated soil-straw mixture on the day of the
165 experimental setting-up (T0) was considered as the starting point.

166 Samples were taken 0 (T0), 1 (T1), 2 (T2), 8 (T8), and 24 (T24) weeks after the setting-up of
167 the experiment. At each sampling time, the earthworms were removed from the microcosms.
168 The whole remaining contents (i.e. straw and soil) were mixed and freeze-dried and ground to
169 powder to make a homogenous mixture of straw and soil, and preserved at -20°C until DNA
170 extraction.

171 **2.5- Microcosms preparation**

172 A transparent solution of DON was obtained by mixing 5 mg of DON powder (Sigma
173 Aldrich, France - CAS No. 51481-10-8) in 4 ml of pure ethanol. The required concentration
174 (66.7 µg ml^{-1}) was prepared by dissolving the transparent solution of DON in sterile water.
175 The water-holding capacity of the straw was found to be 3.5 ml of water in 1 g of straw dry
176 weight (d.w.). The straw was inoculated with *F. graminearum* by spraying and mixing with
177 1.5 ml of the conidial suspension (6.7×10^2 conidia ml^{-1}) per gram of straw (d.w.). Then the
178 straw was divided into two halves: one half was contaminated with DON by spraying and
179 mixing with 1.5 ml of DON solution (66.7 µg ml^{-1}) per gram of straw (d.w.), while the other
180 half was moistened by spraying and mixing with distilled water to provide homogenous
181 humidity to the whole straw. Finally, the whole straw contained 10^3 *F. graminearum* conidia
182 g^{-1} of straw (d.w.), and the DON-contaminated straw contained DON at a concentration of
183 100 µg g^{-1} of straw (d.w.).
184 Two-L plastic jars (13 cm diameter × 15 cm height) were used as experimental units
185 (microcosms). A hole was made in the lid of each jar to allow earthworms to breathe and let
186 out the gases produced as a result of straw decomposition. This hole was covered with plastic
187 gauze to avoid earthworms escaping.
188 Each microcosm was filled with 1kg of soil (dry weight) and 10 g of *F. graminearum*-infected
189 straw (dry weight) contaminated with DON or not. Ten g of straw contaminated with 10 µg of
190 DON g^{-1} (d.w) were introduced into 1kg of soil (d.w.) to contaminate each microcosm with
191 1µg DON g^{-1} soil-straw mixture, which is close to the threshold limit recommended by the
192 European Union for unprocessed wheat grains (1.25 µg of DON g^{-1}). The straw was either
193 mixed with the soil or placed on its surface to form a layer. Four earthworms for a total mean
194 biomass of 13.7 (± 0.76) g were added to each required microcosm. This number of four
195 earthworms per kg of soil was chosen so that there are enough earthworms for their role on

196 the fate of the straw is measurable and that, possibly, they can reproduce or decrease. At the
197 same time, this number cannot be higher to prevent them from starving to death.

198 **2.6- Quantification of earthworms and nematodes**

199 At the different sampling times, the earthworms previously removed from the soil were
200 washed twice with water to remove their outer soil and then gently surface-dried on tissue
201 paper. They were counted, and the total weight of all the earthworms was measured for each
202 microcosm and termed as their biomass.

203 Nematode extraction and counting were carried out using the elutriator described by
204 Oostenbrink (1960). Briefly, nematodes were extracted from 250 g of soil or soil-straw
205 mixture from each microcosm. The soil or soil-straw mixture was suspended in 1 L of water
206 and sieved to 1 mm to remove the big fragments of straw. The sieved soil suspension
207 containing nematodes was put in the elutriator using an upward flow of water (flow rate: 35 L
208 h^{-1}) for 15 min to accelerate the natural process of sedimentation of the coarse particles, and
209 the soil fine particles were collected and sieved to 50 µm. The contents of the sieve were
210 collected and transferred to a sieve containing a pre-moistened tissue paper. The sieve was
211 placed in a large Petri dish containing an amount of water that could keep the permanent
212 humidification of the sieve and ensure nematode migration. The nematodes migrated below
213 the sieve into the Petri dish. After 48 h, the contents of the Petri dish were poured into a
214 graduated cylinder up to a 25-ml volume. Then 2 ml were spread on a plate grid specific for
215 counting the individuals under a dissecting microscope. This counting was performed twice
216 for each sample. For each sample, 2 ml of the extracted nematode suspension were stored in
217 microtubes at -20°C for DNA extraction.

218 **2.7- DNA extraction**

219 DNA was extracted from different substrates (soil, wheat straw, and soil-straw) following a
220 procedure adapted from Edel-Hermann et al. (2004). Two g of freeze-dried and ground soil or

221 soil-straw mixture or 0.5 g of freeze-dried and ground straw were added to a 15-ml tube
222 containing 4 g of 1.4-mm diameter silica beads, 5 g of 0.1-mm diameter ceramic beads, and
223 eight 2-mm diameter glass beads. Then 8 ml of lysis buffer containing 100 mM Tris HCl (pH
224 8.0), 100 mM EDTA (pH 8.0), 100 mM NaCl, and 2% sodium dodecyl sulfate (w/v) were
225 added to each sample. The samples were shaken for 90 s at 4 m s^{-1} in a Fast Prep-24
226 Instrument (MP Biomedicals, Illkirch, France) and incubated for 30 min at 70°C with mixing
227 after 15 and 30 min. Then they were centrifuged at 7,000 × g for 5 min at 15°C. The
228 supernatants were recovered in 2-ml microtubes and incubated for 10 min on ice with 0.1
229 volume of 5 M potassium acetate. After centrifugation at 14,000 × g for 10 min at 4°C, the
230 nucleic acids in the supernatants were precipitated with one volume of ice-cold isopropanol
231 for 15 min at -20°C. The precipitate was pelleted by centrifugation at 15,000 × g for 20 min at
232 4°C, washed twice with 70% (v/v) ice-cold ethanol, air-dried, and dissolved in 200 µl of 10
233 mM Tris HCl (pH 8.0). Out of 200 µl, only 100 µl of extracts of soil nucleic acids were used
234 for purification, and the other 100 µl were frozen at -20°C for further use if needed.
235 The extracts of soil nucleic acids were purified twice by using a polyvinylpolypyrrolidone
236 (PVPP) spin column to remove co-extracted humic acids, as described by Edel-Hermann et al.
237 (2004). Then, the DNA extract was purified using a GeneClean Turbo kit (MP Biomedicals).
238 DNA extractions from the nematode-water suspensions were carried out by using the same
239 procedure as the one used for the soil, with the following exceptions: 2 ml of nematode-water
240 suspension were used instead of 2 g of soil, and 2 ml of 2-fold more concentrated lysis buffer
241 were used for nucleic acid extraction. The dry DNA was dissolved in 30 µl of 10 mM Tris
242 HCl pH 8.0 and purified using a GeneClean Turbo kit.
243 Ten-µl aliquots of purified DNA extracts were resolved by electrophoresis in a 0.8% agarose
244 gel together with dilutions of calf thymus DNA (Bio-Rad). The gels were stained with
245 ethidium bromide, photographed under a camera, and the staining intensities were measured

with Bio-1D^{++} software (Vilber-Lourmat, Marne-la-Vallée, France). The DNA concentrations were calculated using a standard curve of 25-150 ng of calf thymus DNA plotted versus the staining intensity. DNAs were stored at -20°C until use.

2.8- DON extraction and quantification

The fate of DON during straw incorporation to the soil was monitored up to 24 weeks after the start of the experiment. DON was extracted and quantified at T = 0, 1, 2, 8, and 24 weeks for each treatment in the presence of earthworms, with the straw left on the soil surface (SSE and SSDE) or incorporated to the soil (SME and SMDE). It was also extracted and quantified at T = 0, 8, and 24 weeks in the absence of earthworms when the straw was left on the soil surface and contaminated (SSD) or not (SS) with DON (Table 1). The procedure was the one used by Bily et al. (2004) and recently adapted by Leplat et al. (2018). Briefly, 2 g of each sample were weighed and transferred to 50-ml tubes with 10 ml of an acetonitrile/ultrapure H_2O mixture (84/16). Samples were shaken at 230 rpm for 3 h at 25°C before centrifugation (10 min, 4,500 rpm) to allow DON to transfer from the matrix to water. The supernatant was purified using an Immuno Affinity Column (IAC) Trichothecene P (R-Biopharm Rhône Ltd., Saint-Didier-au-Mont-d'Or, France). The supernatant was eluted by gravity and DON was retained by anti-DON antibodies contained in the column gel. The retained mixture was washed twice with 3 ml of sterilised ultrapure water using gravity. Elution was done with 1.5 ml of 100% pure methanol (MeOH). The organic phase was evaporated to dryness and dissolved in 200 µl of MeOH/ultrapure H_2O (1 / 1) by vortexing vigorously for 1 min. The suspended residue was filtered through a 0.45–µm PFTE membrane (Merck Millipore, Billerica, USA). The extracts were maintained at -20°C until analysis by high-performance liquid chromatography with diode-array detection (HPLC-DAD).

DON detection and quantification was carried out using a Beckman Gold® HPLC system equipped with a programmable pump module 126 coupled to a 168–nm photodiode array

detector (Beckman Coulter, Fullerton, USA). An Ultrasphere® Octyl Analytical reverse-phase column (4.6 x 150 mm; particle size 5 µm) was used as an analytical column, and then a Pre-Column Ultrasphere® Octyl Guard (4.6 x 45 mm; particle size 5 µm) was used. The chromatographic system contained ultrapure H_2O at pH 2.6 with H_3PO_4 (eluent A) and acetonitrile (eluent B). The gradient started with 5% B. From 0 to 14 min, it linearly increased to 30% B, and then another linear increase to 90% B in the next 2 min. This phase was kept up to 18 min. The gradient was then linearly decreased to 5% B in 2 min. Finally, the column was equilibrated for 4 min before the next injection. The flow rate was 1 ml min^{-1}. The injection volume was 20 µl. The detection wavelength was 220 nm and the retention time was 8.32 min.

A calibration curve was prepared by spiking a matrix extract with a standard solution of DON. The detection limit was determined as the concentration with a signal-to-noise ratio of 3:1. The quantification limit was at a signal-to-noise ratio of 10:1. The DON standard (ref M125S100MEVC) was purchased from Cluzeau Info Labo (C.I.L.) (33220 Sainte-Foy-La-Grande, France; https://shop.cluzeau.fr/).

2.9- Quantification of *Fusarium graminearum*, fungi, and bacteria

Fusarium graminearum and overall fungal and bacterial densities were quantified in all the samples using SYBR Green dye-based real-time Q-PCR carried out on an ABI PRISM 7900 HT Sequence Detection System (Applied Biosystems TM, USA).

Fusarium graminearum was quantified by using the specific primer pair Fg16NF (ACA GAT GACAAGATTCAGGCACA)/Fg16NR (TTCTTTGACATCTGTTCAACCCA) (Nicholson et al., 1998), which amplifies a 284-bp fragment. One µl of DNA was mixed with 0.25 µM of each primer, 1 µg of T4 gene 32 protein (MP Biomedicals), 6.5 µl of Q-PCR SYBR Green ROX MIX (2X) (Thermo Fisher scientific Inc., USA), and DNAse-RNAse-free water up to a final volume of 13 µl. The real-time Q-PCR conditions consisted of an initial step of 10 min

296 at 95°C for enzyme activation, followed by 45 cycles of 15 s at 95°C (denaturation), 30 s at
297 64°C (annealing), 30 s at 72°C (elongation), and 30 s at 78°C (data acquisition). Then a
298 melting curve analysis was performed as follows: 95°C for 30 s, 70°C for 30 s, and then the
299 temperature was increased from 70 to 95°C at a 2 % (°C min^{-1}) ramp rate.
300 Fungal quantification was carried out by targeting a fungus-specific 348-bp fragment of 18S
301 rDNA using the primer set FR1 (AICCATTCAATCGGTAIT)/FF390
302 (CGATAACGAACGAGACCT) (Prévost-Bouré et al., 2011; Vainio and Hantula, 2000). For
303 the PCR, 1 µl of DNA was mixed with 0.25 µM of each primer, 0.5 µg of T4 gene 32 protein,
304 6.5 µl of QPCR SYBR Green ROX MIX (2X), and DNAse-RNAse-free water up to a final
305 volume of 13 µl. The real-time Q-PCR conditions consisted of an initial step of 10 min at
306 95°C, followed by 40 amplification cycles with 15 s at 95°C (denaturation), 30 s at 50°C
307 (annealing), and 60 s at 72°C (elongation and data acquisition). Then a melting curve analysis
308 was performed with cycling set as follows: 95°C for 15 s, 70°C for 15 s, and then a
309 temperature increase from 70 to 95°C at a 2 % (°C min^{-1}) ramp rate.
310 The bacterial quantifications were performed by targeting a bacterium-specific 194-bp
311 fragment of 16S rDNA using the primers 341F (CCTACGGGAGGCAGCAG) and 534R, also
312 referred to as 515R, (ATTACCGCGGCTGCTGGCA) (López-Gutiérrez et al., 2004). One µl
313 of DNA was mixed with 0.25 µM of each primer, 0.5 µg of T4 gene 32 protein, 7.5 µl of Q-
314 PCR SYBR Green ROX MIX (2X) and DNAse-RNAse-free water up to a final volume of 13
315 µl. The real-time Q-PCR conditions consisted of an initial step of 10 min at 95°C for enzyme
316 activation, a second step corresponding to the 35 PCR cycles with 15 s at 95°C (denaturation),
317 30 s at 60°C (annealing), and 30 s at 72°C (elongation and data acquisition). Then a melting
318 curve analysis was performed with cycling set as follows: 30 s at 95°C, 30 s at 72°C, and then
319 a temperature increase from 72°C to 95°C at a 2 % (°C min^{-1}) ramp rate.

Three technical replicates were performed for each biological sample of each of the three communities. A standard curve based on cycle threshold (Ct) values vs. known quantities of target DNA was generated for each real-time Q-PCR assay by using ten-fold dilution series of plasmid DNA containing the cloned specific region for each community corresponding to 10^9 to 10^2 copies of target DNA per PCR reaction. Two repeats of the standard curve were assessed during each replication. The curve was used to quantify the amount of target DNA in the different DNA samples. The assay had a linear range of quantification, and there was a good correlation between the Ct values and the concentrations of cloned DNA (r^2 = 0.8373, 0.9629, and 0.9629 for *F. graminearum,* fungi, and bacteria, respectively). Results were expressed as numbers of copies g^{-1} soil (d.w.).

Additionally, densities of culturable fungi and bacteria were estimated as colony-forming units (CFU) on the appropriate media. Briefly, 5 g of soil or soil-straw mixture were added to 45 ml of sterile water and shaken for 20 min in a three-dimensional shaker at 700 oscillations min^{-1}. Ten-fold dilutions were made for each sample from the stock suspension. Fungi were quantified on MEA (malt extract 10 g L^{-1}, agar 15 g L^{-1}) supplemented with citric acid (250 mg L^{-1}), and antibacterial antibiotics (chlortetracycline 50 mg L^{-1} and streptomycin sulphate 100 mg L^{-1}). Bacteria were quantified on YPGA (yeast extract 5 g L^{-1}, peptone 5 g L^{-1}, glucose 10 g L^{-1}, agar 15 g L^{-1}) supplied with cycloheximide (50 mg L^{-1}). Three replicates for bacteria and 5 replicates for fungi were performed.

2.10- Fungal, bacterial, protozoan, and nematode community structures

The structural changes in the microbial and microfauna communities during straw decomposition were determined by using terminal restriction fragment length polymorphism (T-RFLP) analysis.

Fungal community structures were assessed by targeting the internal transcribed spacer (ITS) of the ribosomal DNA (rDNA) with primers ITS1F (CTTGGTCATTTAGAGGAAGTAA)

345 (Gardes and Bruns, 1993) and ITS4 (TCCTCCGCTTATTGATATGC) (White et al., 1990).
346 Primer ITS1F was 5′-end-labeled with the fluorescent dye D3 (Beckman Coulter, Fullerton,
347 CA, USA). PCR amplifications were performed in a final volume of 25 µl by mixing 20 ng of
348 DNA with 0.2 µM of each primer, 200 µM each of dATP, dCTP, dGTP, and dTTP, 6 U of
349 *Taq* DNA polymerase (MP Biomedicals), 0.16 ng ml^{-1} of bovine serum albumin (BSA), and
350 PCR reaction buffer containing 2 mM $MgCl_2$. DNA amplifications were performed in a
351 Mastercycler (Eppendorf, Hamburg, Germany) with an initial denaturation of 5 min at 94°C
352 followed by 35 cycles of denaturation (30 s at 94°C), primer annealing (30 s at 55°C), and
353 extension (1 min at 72°C), and a final extension of 10 min at 72°C.
354 Bacterial community structures were assessed by targeting the 16S rDNA with primers 27F
355 (AGAGTTTGATCCTGGCTCAG) (Edwards et al., 1989) and 1392R
356 (ACGGGCGGTGTGTACA) (Braker et al., 2001). Primer 27F was 5′-end-labelled with the
357 fluorescent dye D3 (Beckman Coulter). PCR amplifications were performed in a final volume
358 of 25 µl containing 20 ng of DNA with 0.2 µM of each primer, 200 µM of dNTP, 6 U of *Taq*
359 DNA polymerase, and PCR reaction buffer containing 1.5 mM $MgCl_2$. DNA amplifications
360 were performed in a Mastercycler with an initial denaturation of 3 min at 94°C followed by
361 30 cycles of denaturation (1 min at 94°C), primer annealing (1 min at 57°C), and extension (1
362 min at 72°C), and a final extension of 10 min at 72°C.
363 In the case of the protozoan community structure, the amplification was performed using the
364 group-specific PCR primers Kin24SF (TAGGAAGACCGATAGCGAACAAGTAG) 5′-end-
365 labelled with the fluorescent dye D3 and Kin24SR (TTTCGGGTCCAAACAGGCACACT),
366 which target the 24S rDNA of the flagellate kinetoplastids (Rasmussen et al., 2001). This
367 group includes bacterivorous and fungivorous flagellates and is sensitive to the introduction
368 of exogenous molecules in the soil (Ekelund et al., 2000). PCR amplifications were performed
369 in a final volume of 25 µl by mixing 20 ng of DNA sample with 0.2 µM of each primer, 400

370 μM of each dNTP, 6 U of *Taq* DNA polymerase, 0.16 ng ml^{-1} of BSA, and PCR reaction
371 buffer containing 3 mM $MgCl_2$. DNA amplifications were performed in a Mastercycler with
372 an initial denaturation step of 3 min at 94°C followed by 35 cycles of denaturation (1 min at
373 94°C), primer annealing (1 min at 60°C), and extension (1 min at 72°C), and a final extension
374 of 5 min at 72°C.
375 Nematode community structures were characterised using the specific primers NEMF1
376 (CGCAAATTACCCACTCTC) 5′-end-labelled with the fluorescent dye D3, and S3
377 (AGTCAAATTAAGCCGCAG), which target the 18S rDNA gene (Waite et al., 2003). PCR
378 amplifications were performed in a final volume of 25 μl by mixing 20 ng of soil DNA with
379 0.3 μM of each primer, 250 μM of each dNTP, 6 U of *Taq* DNA polymerase, 16 ng ml^{-1} of
380 BSA, and PCR reaction buffer containing 2 mM $MgCl_2$. DNA amplifications were performed
381 in a Mastercycler with an initial denaturation step of 3 min at 94°C followed by 30 cycles of
382 denaturation (1 min at 94°C), primer annealing (1 min at 53°C), and extension (1 min at
383 72°C), and a final extension of 10 min at 72°C.
384 PCR products were controlled by electrophoresis in 2% agarose gels for fungi, protozoa and
385 nematodes, and 1% agarose gels for bacteria. The PCR products were purified by using a
386 MinElute PCR purification kit (Qiagen, Courtaboeuf, France) according to the manufacturer's
387 instructions, with two final elutions of the PCR products in 2 × 10 μl. Purified amplicons were
388 quantified by comparison with known quantities of the molecular-weight marker Smart
389 Ladder (Eurogentec, Seraing, Belgium) in 2 % agarose gels for fungi, protozoa and
390 nematodes, and 1 % agarose gels for bacteria. Then, 120 ng of purified amplicons were
391 digested with 5 U of restriction enzyme in a final volume of 100 μl. The restriction enzymes
392 that yielded the greatest diversity were used for each community, i.e. *Hinf*1 for fungi, *Hae*III
393 for bacteria, *Alu*I for protozoa, and *Taq*I for nematodes. The digestion reactions were
394 incubated for 3 h at 37°C, except for *Taq*I at 65°C.

395 The digested products were precipitated with 2 µl of 2.5 mg ml^{-1} of glycogen (Beckman
396 Coulter), 10 µl of 3 M sodium acetate (pH 5.2), and 250 µl of ice-cold ethanol, and were
397 centrifuged for 15 min at 12,000 × g at 4°C. The digested DNAs were rinsed twice with 200
398 µl of ice-cold 70% ethanol, and air-dried. The DNAs were re-suspended in 63 µl of sample-
399 loading solution (SLS, Beckman Coulter) and mixed with 30 µl of a mixture containing 28.8
400 µl of SLS and 1.2 µl of 600-bp size standard (Beckman Coulter). For each sample 30 µl were
401 deposited in three replicates in the wells of a 96-well plate and loaded onto a capillary
402 electrophoresis sequencer CEQ™ 8000 (Beckman Coulter).
403 Analyses were run with the Frag 4-30 s method including a denaturation step of 2 min at
404 90°C, injection at 2,000 V for 30 s, and separation at 4,800 V for 70 min. After
405 electrophoresis, the length and the signal intensity of the fluorescently labelled terminal
406 restriction fragments (TRF) were automatically calculated by comparison with the size
407 standard using the CEQ 8000 Genetic Analysis System version 8.0.52. The 60 to 640 bp
408 fragments corresponding to the size range of the standard were considered. The comparison of
409 the TRF sizes between samples was automated by assigning them to discrete categories using
410 the program Lis with an interval of 1.25 bp (Mougel et al., 2002). The T-RFLP analyses were
411 performed in triplicate for each PCR product. Mean values for the intensity of the peaks found
412 in at least two of the three analyses were considered for further statistical analyses of the
413 microbial community structure.
414 **2.11- Statistical analyses**
415 DON amounts in the different compartments over time, *F. graminearum*, fungal, and bacterial
416 molecular biomass, as well as fungal, bacterial, earthworm, and nematode densities were
417 compared between treatments and sampling times by analysis of variance (ANOVA) and
418 Fisher LSD test (p=0.05) using XLSTAT-Pro version 7.1 (Addinsoft).

419 The communities characterised based on their TRF size and intensity measured from peak
420 heights were compared by principal component analysis (PCA) using ADE-4 software
421 (Thioulouse et al., 1997). PCA results were displayed as variations on a two-dimensional
422 diagram for each community. The resulting significance of the structure was checked using
423 Monte-Carlo tests ($p < 0.05$) with 1,000 random permutations of the data.
424
425 **3- Results**
426 **3.1- Fate of DON in wheat straw inside the soil**
427 The fate of DON during straw incorporation in the soil was monitored up to 24 weeks after
428 the start of the experiment. At T0, 0.831 µg of DON g^{-1} of soil-straw mix was detected (Table
429 1),
430 i.e. 83% recovery of the amount added to the system, either due to adsorption on the matrix or
431 inefficient extraction. Anyway, relatively to the initial value at T0, DON disappeared very
432 rapidly when the straw was incorporated in the soil. At T1, the amount of DON was
433 approximately half (0.349 ± 0.034 µg g^{-1}) that of T0 (0.831 µg g^{-1}) in SMDE samples. The
434 quantity of DON became significantly lower (0.033 ± 0.02 µg g^{-1}) at T2 and soon went below
435 the quantification limit after T2. On the other hand, DON disappearance was very slow when
436 the DON-contaminated straw was left on the soil surface (SSD and SSDE). DON started
437 disappearing after 2 weeks, and was very low after 8 weeks (0.012 ± 0.00 µg g^{-1}) and not
438 detected at 24 weeks.
439 Surprisingly enough, a low quantity of DON (0.105 ± 0.02 µg g^{-1}) was also found at T1 in the
440 SSE microcosms (no DON, straw on the soil surface). It disappeared and dropped below the
441 detection limit at T2. DON traces (below the limit of quantification) were found in non-
442 contaminated straw (without DON addition) incorporated in the soil (SME).

443 Comparing DON quantities at T = 8 and 24 with DON-contaminated straw on the soil surface
444 in the presence or absence of earthworms showed a link between the presence of earthworms
445 and decreased DON quantities in the system. At T8, a significantly higher amount of DON
446 (0.400 ± 0.09 µg g^{-1}) was found in the absence of earthworms (SSD) than in the presence of
447 earthworms (SSDE) (0.012 ± 0.00 µg g^{-1}). DON dropped below the quantification limit
448 (0.0018 µg g^{-1} of soil-straw mixture) or detection limit (0.00034 µg g^{-1} of soil-straw mixture)
449 in all treatments after 24 weeks.

450 **3.2- Impact of DON on the microbial and fauna densities**

451 Densities were compared in the presence or absence of DON when the straw was left at the
452 surface or incorporated in the soil in the presence of earthworms (SSDE, SSE, SMDE and
453 SME) at T = 1, 2, 8, and 24 weeks, and in the absence of earthworms (SSD and SS) at T = 8
454 and 24 weeks.

455 **3.2.1 - *Fusarium graminearum* biomass in relation to DON**

456 The overall *F. graminearum* molecular biomass increased as early as T1, and then decreased
457 with time (Fig. 1A). *F. graminearum* growth was particularly stimulated in the SMDE
458 microcosms in week 1. Later, no impact of the different treatments was observed until week
459 8. DON was found to support *F. graminearum* growth in week 24 in SMDE microcosms that
460 contained DON-contaminated straw incorporated in the soil.

461 The DON-contaminated straw had no impact on *F. graminearum* biomass when the straw was
462 left on the soil surface in the presence (SSDE) or absence (SSD) of earthworms throughout
463 the experiment.

464 **3.2.2 -Effect of DON on fungal and bacterial biomass**

465 Fungal and bacterial biomass increased after the microcosms were set up and then decreased
466 from T2 until the end of the experiment (Fig. 1B and 1C). At T1, fungal densities were
467 significantly higher in microcosms that incorporated straw in the soil (SME, SMDE).

Moreover, fungal as well as bacterial biomass values were significantly higher in the SMDE (DON-contaminated) microcosms than in the other microcosms. At T2, their biomass values significantly plummeted in the SMDE microcosms as compared to the other microcosms. This negative impact was still observed at T8. However, at T24, fungal and bacterial biomass values were again higher in the microcosms with DON-contaminated straw incorporated in the soil (SMDE) as compared to SME microcosms. On the other hand, the DON-contaminated straw left on the soil surface had no impact on fungal and bacterial biomass values in the presence of earthworms (SSDE) throughout the experiment. Fungal biomass was higher in the absence of earthworms (SSD) than in all other treatments at T24, while there was no difference at T8. Bacterial biomass was not affected by the DON-contaminated straw left at the soil surface in the presence (SSD) or absence (SSDE) of earthworms.

Densities of culturable microorganisms were also evaluated (Fig.2A and Fig.2B). Until T24, there was no significant effect of DON on the fungal and bacterial culturable densities whether DON-contaminated straw was incorporated in the soil (SMDE) or left at its surface (SSDE and SSD) in the presence or in the absence of earthworms. At T24, the fungal density is lower in the microcosms in which DON was present (SMDE and SSDE) compared to those in which there was no DON (SNE and SSE). Globally, the culturable fungal density increased after two weeks, unlike total molecular densities, but was not different from those at T0 and T24 (Fig. 2A). As for fungi, at T24, the bacterial density was lower in the microcosms in which DON-contaminated straw was left at the soil surface (SSDE) compared to those in which non-contaminated straw was left at the soil surface. Globally, the culturable bacterial density increased in the first week and then decreased over time (Fig. 2B), which was more or less the same as total molecular biomass.

3.2.3 -Effect of DON on earthworm and nematode densities

492 The presence of DON had no impact on earthworm biomass (Fig. 2C). The cumulative
493 earthworm biomass decreased remarkably up to T2 and then along with the disappearance of
494 straw until T24. This drop in biomass was not related to the presence or absence of DON but
495 seems to have been more dependent on food depletion. At T24, new-born earthworms were
496 found in all the treatments, which indicates that earthworm reproduction or cocoon hatching
497 was not affected by the presence of the mycotoxin. A higher number of new-born earthworms
498 was found when DON-contaminated straw was left at the soil surface (SSDE) as compared to
499 non-contaminated straw (SSE). Four new-born earthworms per microcosm (0.12 ± 0.04 g)
500 were found in SSDE microcosms on average, vs. only 1 (0.045 g) from the three biological
501 replicates of the SSE microcosms. Conversely, no significant impact was observed when the
502 straw was initially incorporated in the soil. Five new-born earthworms (0.174 ± 0.053 g) per
503 microcosm were observed in the SMDE microcosms on average, and 4 (0.503 ± 0.52 g) in the
504 SME microcosms. No earthworm died in the first 8 weeks, but mortality was observed at T24,
505 and was slightly higher in the presence of DON. Earthworm mortality in the SSDE
506 microcosms (straw left on the soil surface in the presence of DON) was 41 %, it was 33 % in
507 the same DON-free microcosms (SSE), while it was 12 % in the SMDE microcosms (straw
508 incorporated in the soil in the presence of DON) and 0 % in the absence of DON (SME).
509 The number of nematodes ($2.4 \times 10^3 \pm 3.9 \times 10^2$) in the soil at the time of the experimental
510 setting-up was considered as the starting point (T0) for all the treatments (Fig. 2D). The
511 numbers of nematodes in all microcosms increased soon after T0, and the presence of DON had
512 no influence on the nematode density in the first two weeks. Overall, a negative trend was
513 observed on the nematode community in the presence of DON at T8. This trend became
514 significant at T24 in the SSDE and SSD microcosms. Moreover, earthworms were also found to
515 have suppressed nematodes at T24.

516 **3.3- Structural changes in the microbial and faunal communities**

517 The mean number of TRFs per soil sample was 69 for fungal communities, 115 for bacterial
518 communities, 85 for protozoa communities, and 98 for nematode communities. The presence
519 of DON in the system affected soil microbial communities according to the type of
520 community and the location of the DON-contaminated wheat straw, i.e. whether it was
521 incorporated in the soil or left at its surface.
522 The fungal community structure changed over time from T1 to T8 mainly when the straw was
523 left at the surface, while it remained almost constant over time when the straw was
524 incorporated in the soil (Fig. 3A). It is noticeable that the fungal community structure as a
525 whole was not directly affected by the presence of DON, whether the straw was left at the soil
526 surface or incorporated in it. In the same way, it is difficult to illustrate the direct role of
527 earthworms on the fungal community structure, but a DON-earthworm interaction seems to
528 have affected the fungal community along the first axis when the straw was left at the soil
529 surface.
530 The bacterial community structure evolved over time in all six treatments (Fig. 3B). The
531 presence of DON-contaminated straw had a significant effect on the bacterial community
532 structure immediately after T0 and during the first two weeks, whether the straw was left at
533 the soil surface or incorporated in it. Then, the differences in bacterial community structures
534 started disappearing, and at T8, the differences between DON-contaminated and non-
535 contaminated treatments had disappeared: DON disappeared or was degraded in the first
536 eight weeks in the presence of earthworms (Table 1). The straw location, i.e. left at the soil
537 surface (SSE and SSDE) or incorporated in it (SME and SMDE) only played a significant role
538 on the community structure during the first two weeks. The comparison among the treatments
539 in the presence or in the absence of DON when the straw was left at the soil surface in the
540 presence or in the absence of earthworms (SS, SSE, SSD and SSDE) at T8 and T24 showed
541 that the presence of the earthworms rather than DON modified the bacterial community

structure. The effect of DON in the SSD microcosms (straw was left at the soil surface in the absence of earthworms) was not clear throughout the experiment. Conversely, the community structures at T8 differed in the presence (SSDE) or absence (SSD) of earthworms when the straw was contaminated with DON. However, this effect was no longer found after 24 weeks.

The protozoan community structure in the treatments with straw at the soil surface showed more variability in the biological replicates (Fig. 13C). The presence of DON showed a clear impact on the protozoan community structure throughout the experiment when the straw was left on the soil surface both in the presence and in the absence of earthworms (SSD and SSDE). DON did not affect the protozoan community structure when the straw was incorporated in the soil. The presence of earthworms had no significant impact on the protozoan community structure in the treatments with the straw left at the soil surface in the presence (SSE and SSDE) or absence (SS and SSD) of earthworms at T8 and T24.

Changes in the nematode community structures were more explained by time than by DON (Fig. 3D). The nematode communities remained unchanged in the first two weeks, but then they changed along with the time factor, and this change continued till the end of the six months. The nematode community structure was not affected by the addition of straw in or on the soil until T24. However, at T24, it seems that the straw supply participated in the structuration of the nematode community along the axis 2, and this structure is more marked when the straw was incorporated in the soil than when it was left at the soil surface. Finally, the nematode community structure showed more variability in the biological replicates at the end of the experiment than in the first two weeks.

4- Discussion

564 The fate of DON in winter wheat straw during the saprophytic survival of *F. graminearum*
565 was monitored for up to six months in microcosms where the straw was left at the soil surface
566 or incorporated in it in the presence of the whole soil biota.
567 DON disappeared from all the microcosms within the six months. Interestingly, the rate of
568 disappearance of DON was considerably higher when the straw was incorporated in the soil
569 than when it was left at its surface thanks to a greater exchange surface between the substrate
570 and microorganisms leading to faster straw decomposition (Lupwayi et al., 2004) and
571 microbial DON degradation (Ji et al., 2016; Vanhoutte et al., 2016). More specifically, *F.*
572 *graminearum* showed the same kind of behaviour as the global fungal and bacterial
573 communities and reacted to the presence of DON only where the straw was incorporated in
574 the soil. *Fusarium graminearum* did not produce DON *in situ* or produced it in very low
575 quantities that disappeared very rapidly during the saprophytic survival of the fungus.
576 *Fusarium graminearum* was found to benefit from the presence of DON, and its biomass
577 increased like the overall biomass of the fungal and bacterial communities. Contrary to other
578 fungal populations in the fungal communities, the presence of DON had no negative effect on
579 *F. graminearum* density as compared to the control. Similar results were observed with *F.*
580 *culmorum* (Sarrocco et al., 2012). Although chemical processes partly explain DON
581 disappearance (He et al., 2010; Guo et al., 2020), they are essentially biotic mechanisms
582 especially involving the soil microflora and probably also the soil microfauna, which are
583 suspected in the present study. Mycotoxin-degrading microorganisms including some able to
584 use DON as a carbon source have indeed already been isolated from animal digestive tracts
585 (Schatzmayr et al., 2006; He J et al., 2010; Vanhoutte et al., 2017). These are often anaerobic
586 bacteria, and one of them, *Eubacterium* sp. strain BBSH 797, is marketed to detoxify cereals
587 incorporated into animal diets (Karlovsky, 2011). However, other microorganisms have also
588 been isolated from wheat heads (Ikunaga et al., 2011; Ito et al., 2012) and soil (He C.H. et al.,

589 2008; Sato et al., 2012, Ji et al., 2016; Vanhoutte et al., 2017). The modes of action of the
590 various isolated microorganisms are related to degradation (Garda-Buffon and Badiale-
591 Furlong, 2010; Sato et al., 2010; Ito et al., 2012; Ji et al., 2016) and / or detoxification by
592 transforming DON into other smaller or non-toxic molecules (Völkl et al., 2004; Zhou et al.,
593 2008; He J et al., 2010; McCormick, 2013). Mostly bacteria have been isolated, but fungi are
594 also involved in DON degradation. For instance, *Aspergillus oryzae* and *Rhizopus oryzae*
595 degraded 74 and 90 % of DON in 96 and 240 hours, respectively, in controlled conditions
596 (Garda-Buffon and Badiale-Furlong, 2010). However, their degradation rate is probably lower
597 under natural conditions, and although no microorganism has been isolated, several soil-borne
598 microbial species likely produce mycotoxin-degrading extracellular enzymes, which explains
599 the rapid disappearance of DON in our microcosms (McCormick, 2013). Furthermore,
600 earthworms played a significant role in DON degradation. At T8, the DON concentration in
601 the presence of earthworms was 40 times lower than in the earthworm-free microcosm with
602 the straw left at the soil surface. This comparative assessment is in agreement with previous
603 demonstrations that earthworms have a specific preference for DON-contaminated straw as
604 compared to non-contaminated straw (Oldenburg et al., 2008; Wolfarth et al., 2011).
605 Although this preference has not yet been explained, the choice may well be related to the
606 earthworm gut microflora, which can degrade DON and therefore provide earthworms with
607 specific trophic resources (Schrader et al., 2009). Actually, *L. terrestris* drives the whole soil
608 microfauna, and subsequently the microflora, in the degradation process (Wolfarth et al.,
609 2016; Meyer-Wolfarth et al., 2017). Although no insects or arthropods have been introduced
610 into microcosms to date, the soil naturally hosts fauna components, including nematodes,
611 enchytreids and small arthropods. These small animals are stimulated by the presence of
612 earthworms and increase microbial activity by breaking down big pieces of straw into small

613 ones and burrowing them, thus increasing the accessibility of the substrate including DON to
614 soil microorganisms (Hattenschwiler et al., 2005).
615 Changes in the community structures were monitored over six months to determine the impact
616 of DON on the soil microbial and microfauna communities when it was present in the wheat
617 straw. Bacterial and protozoan community structures were significantly affected in the
618 presence of DON, while fungal and nematode communities showed a conditional reaction and
619 no reaction to DON, respectively. Whether bacterial and protozoan populations were
620 definitively favoured or suppressed by the presence of DON was not assessed, but the ratio
621 between populations within each community changed; this revealed that some
622 microorganisms are more susceptible to the toxin than others. The impact of the mycotoxin
623 may be positive by favouring populations likely to degrade DON, as mentioned above
624 (Ikunaga et al., 2011; Sato et al 2010); it may also be negative, but to our knowledge no lethal
625 effect of DON on fungi or bacteria has been reported.
626 In the case of fungi, the community structures differed depending on whether the straw was
627 left at the soil surface or incorporated in it. Our results are not contradictory to those obtained
628 by Sarrocco et al., (2012). Indeed, although these authors demonstrated similar taxonomic
629 profiles of fungal communities in DON contaminated residues and in uncontaminated
630 residues buried for 7 days in soil, they did not assess the relative abundance of the different
631 taxa and therefore, it is not known whether the fungal community structures were the same in
632 the different residues they analyzed. In our case, the structure was affected by the presence of
633 DON only in the absence of earthworms. The presence of earthworms globally affected the
634 fungal community structure, as already mentioned (Wolfarth et al., 2016; Meyer-Wolfarth et
635 al., 2017). The presence of earthworms might have masked the effect of DON on the fungal
636 communities. On the one hand, certain fungal species are reported to multiply by degrading
637 DON and other mycotoxins (Gardan-Buffon and Badiale-Furlong, 2010; McCormick, 2013),

638 but the other hand, others declined because they were a choice food for earthworms
639 (Bonkowski et al., 2000; Zirbes et al., 2011).
640 The difference in the bacterial community structures in the presence and absence of
641 earthworms, also corresponding to different amounts of DON in these treatments, should be
642 first attributed to the fauna activity and then to the mycotoxin.
643 The effect of DON on protozoa was related to the straw location. This was very clear
644 throughout the whole experiment when the straw was left on the soil in the presence or in the
645 absence of earthworms over the six months. As DON was no more detectable at this final
646 stage, it can be assumed that the changes DON previously caused on the protozoan
647 community were long-lasting, if not definitive. It would be worth checking for the possible
648 erosion of protozoan diversity. When the straw was incorporated in the soil, the presence of
649 DON had a lower impact on protozoa, probably because of a rapid degradation or
650 detoxification of the mycotoxin by microbial and faunal activity (McCormick, 2013).
651 The results obtained in this study are original; it is the first time that the impact of a
652 mycotoxin on soil protozoa has been highlighted. One study highlighted that protozoa were
653 less able than bacteria to detoxify ochratoxin A in rumen fluid, but better efficacy was
654 observed when bacteria, fungi and protozoa were combined (Mobashar et al., 2012).
655 However, nothing is said about a possible negative impact of mycotoxins on these protists.
656 The nematode communities showed no response to the presence of DON in any treatment
657 throughout the whole period of the experiment, whereas nematodes associated with
658 collembolans and earthworms have been shown to play an important role in DON
659 degradation, and even in the regulation of mycotoxin production by *Fusarium* sp. (Wolfarth et
660 al., 2013; 2015). In our experiment, the community structure only changed over time after two
661 weeks and because of straw supply at T24. Although it is likely that the incorporation and
662 fragmentation of straw by earthworms was a structuring factor of the nematode community,

663 this hypothesis can not be tested in the absence of a SM (Straw incorporated to the soil) type
664 of microcosm.
665 Earthworms are known bio-indicators of soil health and have a great importance in agriculture
666 due to their ability to incorporate and degrade wheat crop residues and organic matter (Friberg
667 et al., 2005; Kennedy et al 2013; Jiang et al 2018). Our results are in agreement with these
668 previous findings, and they more specifically emphasize the role of *L. terrestris* in the
669 degradation and incorporation of straw in the soil. Although the earthworm biomass decreased
670 over time, probably because the food stocks depleted, the positive and original point was that
671 this total biomass was not affected by DON-contaminated straw. On the contrary, DON-
672 contaminated straw was found more attractive for earthworms, which is in agreement with
673 other findings (Oldenburg et al., 2008; Wolfarth et al., 2016).
674 Furthermore, the presence of the new-born earthworms in all the treatments containing DON-
675 contaminated straw showed that DON had no negative effect on earthworm reproduction or
676 cocoon hatching. Even more, the presence of higher numbers of new-born earthworms when
677 DON-contaminated straw was left at the soil surface showed that DON stimulated earthworm
678 reproduction or increased hatching. Counting the number of earthworms at each sampling
679 time revealed that earthworm mortality only occurred at the end of the six months. Overall
680 more earthworms died when the straw was left at the soil surface. This mortality had no
681 significant impact on the overall decrease in earthworm biomass, which did not significantly
682 differ across treatments in the presence or absence of DON.
683 Nematodes are also a very important component of arable soil functioning and are considered
684 as soil quality indicators (Schloter et al., 2003; Kapp et al., 2013; Du Preez et al., 2018). More
685 than the direct role of nematodes in organic matter decomposition and in pathogen control
686 (Hasna et al., 2007), it was the structure of the nematode community impacted by crop
687 residues and the nature of the organic matter that determined the indicator value (Berry et al.,

688 2007). In our soil microcosms, the number of nematodes increased over time during straw
689 decomposition while earthworm biomass decreased. There was no impact of DON on
690 earthworm biomass in the first two weeks, but a negative trend related to the presence of
691 DON was found at T8; it was significant at T24, suggesting a progressive more than a sudden
692 poisoning effect. However, such an effect seems to be variable depending on the nematodes.
693 The developmental process and egg hatching of *Caenorhabditis elegans* were reduced in
694 liquid medium containing DON (Gowrinathan et al., 2011), while the population density of
695 *Aphelenchoides saprophilus* increased when the soil received DON-contaminated wheat straw
696 (Wolfarth et al., 2015). The outcome of nematode - earthworm interactions also seems
697 variable. The nematode densities increased in the microcosms with the decrease in earthworm
698 biomass; such a negative relationship between nematode biomass and earthworm biomass
699 during the decomposition of an organic substrate has already been reported (Dominguez et al.,
700 2003). Conversely, a positive relationship between nematodes, earthworms and collembolans
701 resulted in a control of DON contents in wheat straw in the soil (Wolfarth et al., 2016).
702 The multiple interactions occurring in the studied system could have overshadowed a stronger
703 impact of DON than the one revealed by the community structure fingerprints or the
704 community molecular biomass. On the contrary, the classic microbiological methods revealed
705 some variability among microorganisms in their response to the presence of DON, but they
706 are not accurate enough and cannot ensure that the observed differences can be attributed to
707 DON, to biotic interactions, or to technical limitations. Therefore, although molecular and
708 classic microbiological assessments of the fate of bacteria and fungi in the crop residues in the
709 presence or absence of DON occasionally gave different results, the two techniques are
710 complementary. They both revealed similar trends, the main ones being that the microbial
711 communities, including protozoa, were affected to various extents by the presence of DON
712 but *F. graminearum* was not. All of these results are reassuring since DON present in the crop

residues is rapidly biodegraded without creating definitive disturbances within soil microflora and microfauna and without providing any particular advantage for *F. graminearum* survival. This is why the risk associated with the presence of DON in food and web goes first through the control of *F. graminearum*

5-Conclusion

This study provides a wide overview and a comprehensive knowledge on the fate of DON in crop residues in the soil and its impact on the soil microflora and fauna.

DON did not remain long in the straw and disappeared completely in all the treatments within six months. *F. graminearum* did not produce DON *in situ*, or DON was produced in very low quantities that did not compensate for its disappearance, whether through degradation or transformation into unmeasured molecules. The location of straw played a significant role, as DON disappeared more rapidly when the straw was incorporated in the soil than when it was left at its surface. The presence of earthworms enhanced DON disappearance.

The bacterial and fungal densities were negatively affected when DON-contaminated straw was incorporated in the soil, and when DON was present, it significantly changed the bacterial community structure. The effect on the fungal community structure was seen only in the absence of earthworms. *F. graminearum* was not affected by the presence of DON. In the case of protozoa, straw location was more important, and the effect of DON remained measurable throughout the experiment. The nematode community structure remained unaffected by the presence of DON.

Earthworm biomass was not affected by exposure to DON. Both earthworm reproduction and cocoon hatching were stimulated in the presence of DON when the straw was left on the soil surface. Nematode densities were negatively affected and their numbers were reduced, but the impact appeared late in the experiment.

In short, DON briefly impacted the soil communities, disappeared over time mainly through microbial and fauna activities, and gave no observable advantage to the soil-borne *F. graminearum* population. Thus, of the three hypotheses that our study aimed at testing, the first concerning the competitive advantage provided by DON to *F. graminearum* is refuted while those concerning the palatability of straw for earthworms and microfauna as well as the stimulation of biotic interactions in soil by DON are validated.

Acknowledgments

The first author was financially supported by Higher Education Commission (HEC) of Pakistan under a Franco-Pakistan collaborative agreement from 2009 to 2012. This project, FARINE +, was funded under the 4th Call for Projects FUI 2007 and managed by Pole Vitagora, (the taste, nutrition and health innovative Pole), Dijon-France. The authors are grateful to Dr Hanna Friberg (SLU, Sweden) for the fruitful discussions during the meetings of the M. Abid PhD Steering Committee

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

Bai, G., Shaner, G., 2004. Management and resistance in wheat and barley to Fusarium head blight. Annu. Rev. Phytopathol. 42, 135-161.

760 Bai, G.H., Desjardins, A., Plattner, R., 2002. Deoxynivalenol-nonproducing *Fusarium*
761 *graminearum* Causes Initial Infection, but does not Cause Disease Spread in Wheat
762 Spikes. Mycopathologia 153, 91-98.
763 Barbi, F., Bragalini, C., Vallon, L., Prudent, E., Dubost, A., Fraissinet-Tachet, L., Marmeisse,
764 R., Luis,P., 2014. Pcr primers to study the diversity of expressed fungal genes encoding
765 lignocellulolytic enzymes in soils using high-throughput sequencing. Plos One
766 9:e116264. doi:10.1371/journal.pone.0116264
767 Berry, S., Spaull, V., Cadet, P., 2007. Impact of harvesting practices on nematode
768 communities and yield of sugarcane. Crop Prot. 26, 1239-1250.
769 Beyer, M., Klix, M.B., Verreet, J.A., 2007. Estimating mycotoxin contents of *Fusarium*-
770 damaged winter wheat kernels. Int. J. Food Microbiol. 119, 153-158.
771 Bilska K., Jurczak S., Kulik T., Ropelewska, E., Olszewski, J., Zelechowski, M., Zapotoczny,
772 P., 2018. Species composition and trichothecene genotype profiling of *Fusarium* field
773 isolates recovered from wheat in Poland. Toxins 10, 325, doi:10.3390/toxins10080325
774 Bily, A.C., Reid, L.M., Savard, M.E., Reddy, R., Blackwell, B.A., Campbell, C.M., et al.
775 2004. Analysis of *Fusarium graminearum* mycotoxins in different biological matrices
776 by LC/MS. Mycopathologia, 157, 117–126.
777 Bonkowski, M., Griffiths, B.S., Ritz, K., 2000. Food preferences of earthworms for soil fungi.
778 Pedobiologia 44, 666-676.
779 Bottalico, A., Perrone, G., 2002. Toxigenic *Fusarium* species and mycotoxins associated with
780 head blight in small-grain cereals in Europe. Eur. J. Plant Pathol. 108, 611-624.
781 Bouché, M.B., 1972. Lombriciens de France: écologie et systématique. Institut National de la
782 Recherche Agronomique. France.
783 Braker, G., Ayala-del-Río, H.L., Devol, A.H., Fesefeldt, A., Tiedje, J.M., 2001. Community
784 structure of denitrifiers, Bacteria, and Archaea along redox gradients in Pacific

785 Northwest marine sediments by terminal restriction fragment length polymorphism
786 analysis of amplified nitrite reductase (nirS) and 16S rRNA genes. Appl. Environ.
787 Microbiol. 67, 1893-1901.
788 Buee, M., Reich, M., Murat, C., Morin, E., Nilsson, R.H., Uroz, S., Martin, F., 2009. 454
789 Pyrosequencing analyses of forest soils reveal an unexpectedly high fungal diversity.
790 New Phytol. 184, 449-456.
791 Buerstmayr, H., Ban, T., Anderson, J.A., 2009. QTL mapping and marker-assisted selection
792 for Fusarium head blight resistance in wheat: a review. Plant Breed. 128, 1-26.
793 De Almeida, J.L., Tessmann, D.J., Do Couto, H.T.Z., Fostim, M.L., 2016 Effect of Fusarium
794 head blight on deoxynivalenol levels in whole grain and patent flours from different
795 wheat genotypes. World Mycotoxin J. : 9, 229-236.
796 De Wolf, E., Madden, L., Lipps, P., 2003. Risk assessment models for wheat Fusarium head
797 blight epidemics based on within-season weather data. Phytopathology 93, 428-435.
798 Deacon, L.J., Janie Pryce-Miller, E., Frankland, J.C., Bainbridge, B.W., Moore, P.D.,
799 Robinson, C.H., 2006. Diversity and function of decomposer fungi from a grassland
800 soil. Soil Biol. Biochem. 38, 7-20.
801 Dominguez, J., Parmelee, R., Edwards, C., 2003. Interactions between *Eisenia andrei*
802 (Oligochaeta) and nematode populations during vermicomposting. Pedobiologia 47,
803 53-60.
804 Du Preez, G.C., Daneel, M.S., Wepener, V., Fourie, H., 2018.. Beneficial nematodes as
805 bioindicators of ecosystem health in irrigated soils. Appl. Soil Ecol. 132, 155-68.
806 Edel-Hermann, V., Dreumont, C., Pérez-Piqueres, A., Steinberg, C., 2004. Terminal
807 restriction fragment length polymorphism analysis of ribosomal RNA genes to assess
808 changes in fungal community structure in soils. FEMS Microbiol. Ecol. 47, 397-404.

809 Edwards, U., Rogall, T., Blöcker, H., Emde, M., Böttger, E.C., 1989. Isolation and direct
810 complete nucleotide determination of entire genes. Characterization of a gene coding
811 for 16S ribosomal RNA. Nucleic Acids Research 17, 7843-7853.
812 Ekelund, F., Westergaard, K., Soe, D. 2000. The toxicity of the fungicide propiconazole to
813 soil flagellates. Biol. Fertil. Soils. 31, 70-77.
814 Fokunang, C., Tembe-Fokunang, E., Tomkins, P., Barkwan, S., 2006. Global impact of
815 mycotoxins on human and animal health management. Outlook Agric. 35, 247-253.
816 Foroud, N.A., Eudes, F., 2009. Trichothecenes in cereal grains. Int. J. Mol. Sci. 10, 147-173.
817 Friberg, H., Lagerlöf, J., Rämert, B., 2005. Influence of soil fauna on fungal plant pathogens
818 in agricultural and horticultural systems. Biocontrol Sci. Technol. 15, 641-658.
819 Garda-Buffon, J., Badiale-Furlong, E., 2010. Kinetics of deoxynivalenol degradation by
820 *Aspergillus oryzae* and *Rhizopus oryzae* in submerged fermentation. J. Braz. Chem.
821 Soc. 21, 710-714.
822 Gardes, M., Bruns, T., 1993. ITS primers with enhanced specificity for basidiomycetes-
823 application to the identification of mycorrhizae and rusts. Mol. Ecol. 2, 113-118.
824 Gowrinathan, Y., Pacan, J., Hawke, A., Zhou, T., Sabour, P., 2011. Toxicity assay for
825 deoxynivalenol using *Caenorhabditis elegans*. Food Addit. Contam. Part A Chem.
826 Anal. Control Expo. Risk Assess. 28, 1235.
827 Guo, H.Y., Ji, J., Wang, J. S., Sun, X.L., 2020. Deoxynivalenol: Masked forms, fate during
828 food processing, and potential biological remedies. Compr. Rev. Food Sci. Food Saf.
829 19, 895-926.
830 Hasna, M., Insunza, V., Lagerlöf, J., Rämert, B., 2007. Food attraction and population growth
831 of fungivorous nematodes with different fungi. Ann. Appl. Biol. 151, 175-182.
832 Hassan, Y.I., Bullerman, L.B., 2009. Wheat bran as an alternative substrate for macroconidia
833 formation by some *Fusarium* species. J. Microbiol. Methods 77, 134-136.

834 Hättenschwiler, S., Tiunov, A.V., Scheu, S., 2005. Biodiversity and litter decomposition in
835 terrestrial ecosystems. Annu. Rev. Ecol. Evol. Syst. 191-218.
836 He, C.H., Fan, Y.H., Liu, G.F., Zhang, H.B., 2008. Isolation and identification of a strain of
837 *Aspergillus tubingensis* with deoxynivalenol biotransformation capability. Int. J. Mol.
838 Sci. 9, 2366-75.
839 He, J., Zhou, T., Young, J.C., Boland, G.J., Scott, P.M., 2010. Chemical and biological
840 transformations for detoxification of trichothecene mycotoxins in human and animal
841 food chains: a review. Trends Food Sci. Technol. 21, 67-76.
842 Hofgaard, I.S., Seehusen, T., Aamot, H.U., Riley, H., Razzaghian, J., Le, V.H., Hjelkrem, A.-
843 G.R., Dill-Macky, R., Brodal, G., 2016. Inoculum Potential of *Fusarium* spp. Relates to
844 Tillage and Straw Management in Norwegian Fields of Spring Oats. Front. Microbiol.
845 7, 556. doi: 10.3389/fmicb.2016.00556
846 Holb, I.J., Heijne, B., Jeger, M.J., 2006. Effects of integrated control measures on
847 earthworms, leaf litter and *Venturia inaequalis* infection in two European apple
848 orchards. Agric. Ecosyst. Environ. 114, 287-295.
849 Ikunaga, Y., Sato, I., Grond, S., Numaziri, N., Yoshida, S., Yamaya, H., Hiradate, S.,
850 Hasegawa, M., Toshima, H., Koitabashi, M., 2011. *Nocardioides* sp. strain WSN05-2,
851 isolated from a wheat field, degrades deoxynivalenol, producing the novel
852 intermediate 3-epi-deoxynivalenol. Appl. Microbiol. Biotechnol. 89, 419-427.
853 Ito, M., Sato, I., Koitabashi, M., Yoshida, S., Imai, M., Tsushima, S., 2012. A novel
854 actinomycete derived from wheat heads degrades deoxynivalenol in the grain of wheat
855 and barley affected by Fusarium head blight. Appl. Microbiol. Biotechnol. 96, 1059-
856 1070.
857 Janssen, E., Liu, C., Van der Fels-Klerx, H., 2018. *Fusarium* infection and trichothecenes in
858 barley and its comparison with wheat. World Mycotoxin J. 11, 33-46.

859 Ji, C., Fan, Y., Zhao, L., 2016. Review on biological degradation of mycotoxins. Anim. Nutr.
860 2, 127-133.
861 Jiang, Y.B., Wang, J., Muhammad, S., Zhou, A.Q., Hao, R., Wu, Y.P., 2018. How do
862 earthworms affect decomposition of residues with different quality apart from
863 fragmentation and incorporation? Geoderma 326, 68-75.
864 Kapp, C., Storey, S.G., Malan, A.P., 2013. Options for Soil Health Measurement in Vineyards
865 and Deciduous Fruit Orchards, with Special Reference to Nematodes. S. Afr. J. Enol.
866 Vitic. 34, 272-80.
867 Karlovsky, P., 2011. Biological detoxification of the mycotoxin deoxynivalenol and its use in
868 genetically engineered crops and feed additives. Appl. Microbiol. Biotechnol. 91,
869 491-504.
870 Kennedy, T.F., Connery, J., Fortune, T., Forristal, D., Grant, J., 2013. A comparison of the
871 effects of minimum-till and conventional-till methods, with and without straw
872 incorporation, on slugs, slug damage, earthworms and carabid beetles in autumn-sown
873 cereals. J. Agric. Sci. 151, 605-629.
874 Lemmens, M,, Steiner, B., Sulyok, M., Nicholson, P., Mesterhazy, A., Buerstmayr, H., 2016.
875 Masked mycotoxins: does breeding for enhanced Fusarium head blight resistance result
876 in more deoxynivalenol-3-glucoside in new wheat varieties? World Mycotoxin J. 9,
877 741-54.
878 Leplat, J., Friberg, H., Abid, M., Steinberg, C., 2013. Survival of *Fusarium graminearum*, the
879 causal agent of Fusarium head blight. A review. Agron. Sustain. Dev. 33, 97-111.
880 Leplat J., Heraud C., Gautheron E., Mangin P., Falchetto L., Steinberg C., 2016. Colonization
881 dynamic of various crop residues by *Fusarium graminearum* monitored through real-
882 time PCR measurements. J. Appl. Microbiol. 121, 1394-1405.

883 Leplat, J., Mangin, P., Falchetto, L., Heraud, C., Gautheron, E., Steinberg, C., 2018. Visual
884 assessment and computer–assisted image analysis of Fusarium head blight in the field to
885 predict mycotoxin accumulation in wheat grains. Eur. J. Plant Pathol. 150, 1065–1081.
886 López-Gutiérrez, J.C., Henry, S., Hallet, S., Martin-Laurent, F., Catroux, G., Philippot, L.,
887 2004. Quantification of a novel group of nitrate-reducing bacteria in the environment
888 by real-time PCR. J. Microbiol. Methods 57, 399-407.
889 Lupwayi, N., Clayton, G., O'Donovan, J., Harker, K., Turkington, T., Rice, W., 2004.
890 Decomposition of crop residues under conventional and zero tillage. Can. J. Soil Sci.
891 84, 403-410.
892 McCormick, S.P., 2013. Microbial detoxification of mycotoxins. J. Chem. Ecol. 39, 907-918.
893 Megalla SE, Bennett GA, Ellis JJ, Shotwell OI, 1986. Production of deoxynivalenol and
894 zearalenone by isolates of *Fusarium graminearum* Schw. J. Basic Microbiol. 26, 415-
895 419.
896 Mesterházy, Á., 2002. Role of deoxynivalenol in aggressiveness of *Fusarium graminearum*
897 and *F. culmorum* and in resistance to Fusarium head blight. Eur. J. Plant Pathol. 108,
898 675-684.
899 Mesterházy, A., 2014. Breeding for resistance to Fusarium head blight in wheat. In: Leslie, J.
900 F., Logrieco, A.F. (Eds.), Mycotoxin reduction in grain chainseds. Blackwell Science
901 Publication., Oxford, pp. 189-208.
902 Meyer-Wolfarth, F., Schrader, S., Oldenburg, E., Weinert, J., Brunotte, J., 2017. Biocontrol of
903 the toxigenic plant pathogen *Fusarium culmorum* by soil fauna in an agroecosystem.
904 Mycotoxin Res. 33, 237-244.
905 Mishra, S., Tewari, P., Chaudhari, B.P., Dwivedi, P.D., Pandey HP, Das M, 2016.
906 Deoxynivalenol induced mouse skin tumor initiation: Elucidation of molecular
907 mechanisms in human HaCaT keratinocytes. Int. J. Cancer 139, 2033-2046.

908 Mobashar, M., Blank, R., Hummel, J., Westphal, A., Tholen, E., Südekum, K.-H., 2012.
909 Ruminal ochratoxin A degradation—Contribution of the different microbial
910 populations and influence of diet. Anim. Feed Sci. Technol. 171, 85-97.
911 Mougel, C., Thioulouse, J., Perri, G., Nesme, X., 2002. A mathematical method for
912 determining genome divergence and species delineation using AFLP. Int. J. Syst.
913 Evol. Microbiol. 52, 573-586.
914 Mudge, A.M., Dill-Macky, R., Dong, Y., Gardiner, D.M., White, R.G., Manners, J.M., 2006.
915 A role for the mycotoxin deoxynivalenol in stem colonisation during crown rot disease
916 of wheat caused by *Fusarium graminearum* and *Fusarium pseudograminearum*.
917 Physiol. Mol. Plant Path. 69, 73-85.
918 Nicholson, P., Simpson, D.R., Weston, G., Rezanoor, H.N., Lees, A.K., Parry, D.W., Joyce,
919 D., 1998. Detection and quantification of *Fusarium culmorum* and *Fusarium*
920 *graminearum* in cereals using PCR assays. Physiol. Mol. Plant Path. 53, 17-37.
921 Nielsen, L.K., Jensen, J.D., Rodríguez, A., Jørgensen, L., Justesen, A., 2012. TRI12 based
922 quantitative real-time PCR assays reveal the distribution of trichothecene genotypes of
923 *F. graminearum* and *F. culmorum* isolates in Danish small grain cereals. Int. J. Food
924 Microbiol. 157, 384-392.
925 Oldenburg, E., Hoppner, F., Ellner, F., Weinert, J., 2017. Fusarium diseases of maize
926 associated with mycotoxin contamination of agricultural products intended to be used
927 for food and feed. Mycotoxin Res. 33, 167-182.
928 Oldenburg, E., Kramer, S., Schrader, S., Weinert, J., 2008. Impact of the earthworm
929 *Lumbricus terrestris* on the degradation of Fusarium-infected and deoxynivalenol-
930 contaminated wheat straw. Soil Biol. Biochem. 40, 3049-3053.
931 Oostenbrink, M., 1960. Estimating nematode populations by some selected methods.
932 Nematology, 85-102.

933 Paul, P., Lipps, P., Madden, L., 2005. Relationship between visual estimates of Fusarium
934 head blight intensity and deoxynivalenol accumulation in harvested wheat grain: a
935 meta-analysis. Phytopathology 95, 1225-1236.
936 Parry, D., Jenkinson, P., McLeod, L., 2007. Fusarium ear blight (scab) in small grain cereals-a
937 review. Plant Pathol. 44, 207-238.
938 Payros, D., Alassane-Kpembi, I., Pierron, A., Loiseau, N., Pinton, P., Oswald, I.P., 2016.
939 Toxicology of deoxynivalenol and its acetylated and modified forms. Arch. Toxicol. 90,
940 2931-2957.
941 Pereyra, S., Dill-Macky, R., 2008. Colonization of the residues of diverse plant species by
942 *Gibberella zea*e and their contribution to Fusarium head blight inoculum. Plant Dis.
943 92, 800-807.
944 Perez, C., Dill-Macky, R., Kinkel, L.L., 2008. Management of soil microbial communities to
945 enhance populations of *Fusarium graminearum*-antagonists in soil. Plant Soil 302, 53-
946 69.
947 Placinta, C., d'Mello, J., Macdonald, A., 1999. A review of worldwide contamination of
948 cereal grains and animal feed with Fusarium mycotoxins. Anim. Feed Sci. Technol.
949 78, 21-37.
950 Prandini, A., Sigolo, S., Filippi, L., Battilani, P., Piva, G., 2009. Review of predictivemodels
951 for Fusarium head blight and related mycotoxin contamination in wheat. Food Chem.
952 Toxicol. 47, 927-931.
953 Prévost-Bouré, N.C., Christen, R., Dequiedt, S., Mougel, C., Lelièvre, M., Jolivet, C.,
954 Shahbazkia, H.R., Guillou, L., Arrouays, D., Ranjard, L., 2011. Validation and
955 Application of a PCR Primer Set to Quantify Fungal Communities in the Soil
956 Environment by Real-Time Quantitative PCR. PloS one 6, e24166.

957 Rantalainen, M.L., Kontiola, L., Haimi, J., Fritze, H., Setälä, H., 2004. Influence of resource
958 quality on the composition of soil decomposer community in fragmented and
959 continuous habitat. Soil Biol. Biochem. 36, 1983-1996.
960 Rasmussen, L.D., Ekelund, F., Hansen, L.H., Sørensen, S.J., Johnsen, K., 2001. Group-
961 specific PCR primers to amplify 24S a-subunit rRNA genes from Kinetoplastida
962 (Protozoa) used in denaturing gradient gel electrophoresis. Microb. Ecol. 42, 109-115.
963 Rocha, O., Ansari, K., Doohan, F., 2005. Effects of trichothecene mycotoxins on eukaryotic
964 cells: A review. Food Additives and Contaminants 22, 369-378.
965 Rojas, E.C., Jensen, B., Jorgensen, H.J.L., Latz, MaC., Esteban, P., Ding, Y.W., Collinge,
966 D.B., 2020. Selection of fungal endophytes with biocontrol potential against Fusarium
967 head blight in wheat. Biological Control 144, 9.
968 Sarrocco, S., Matarese, F., Moretti, A., Haidukowski, M., Vannacci, G., 2012. DON on wheat
969 crop residues: effects on mycobiota as a source of potential antagonists of *Fusarium*
970 *culmorum*. Phytopathol. Mediterr. 51, 225-235.
971 Sarrocco, S., Valenti, F., Manfredini, S., Esteban, P., Bernardi, R., Puntoni, G., Baroncelli, R.,
972 Haidukowski, M., Moretti, A., Vannacci, G., 2019. Is Exploitation Competition
973 Involved in a Multitrophic Strategy for the Biocontrol of Fusarium Head Blight?
974 Phytopathol. 109, 560-570.
975 Sato, I., Ito, M., Ishizaka, M., Ikunaga, Y., Sato, Y., Yoshida, S., Koitabashi, M., Tsushima,
976 S., 2012. Thirteen novel deoxynivalenol-degrading bacteria are classified within two
977 genera with distinct degradation mechanisms. FEMS Microbiol. Lett. 327, 110-117.
978 Sauvadet, M., Chauvat, M., Fanin, N., Coulibaly, S., Bertrand, I., 2016. Comparing the effects
979 of litter quantity and quality on soil biota structure and functioning: Application to a
980 cultivated soil in Northern France. Appl. Soil Ecol. 107, 261-71.

981 Schatzmayr, G., Zehner, F., Täubel, M., Schatzmayr, D., Klimitsch, A., Loibner, A.P., Binder,
982 E.M., 2006. Microbiologicals for deactivating mycotoxins. Mol. Nutr. Food Res. 50,
983 543-551.
984 Schisler, D., Khan, N., Boehm, M., Slininger, P., 2002. Greenhouse and field evaluation of
985 biological control of Fusarium head blight on durum wheat. Plant Dis. 86, 1350-1356.
986 Schloter, M., Dilly, O., Munch, J., 2003. Indicators for evaluating soil quality. Agric. Ecosyst.
987 Environ. 98, 255-262.
988 Schrader, S., Kramer, S., Oldenburg, E., Weinert, J., 2009. Uptake of deoxynivalenol by
989 earthworms from *Fusarium*-infected wheat straw. Mycotoxin Res. 25, 53-58.
990 Sipila, T.P., Yrjala, K., Alakukku, L., Palojarvi, A., 2012. Cross-site soil microbial
991 communities under tillage regimes: fungistasis and microbial biomarkers. Appl.
992 Environ. Microbiol. 78, 8191-201.
993 Tanaka, T., Hasegawa, A., Yamamoto, S., Lee, U.S., Sugiura, Y., Ueno, Y., 1988. Worldwide
994 contamination of cereals by the *Fusarium* mycotoxins nivalenol, deoxynivalenol, and
995 zearalenone. 1. Survey of 19 countries. J. Agric. Food Chem. 36, 979-983.
996 Tamburic-Ilincic, L., Wragg, A., Schaafsma, A., 2015. Mycotoxin accumulation and
997 *Fusarium graminearum* chemotype diversity in winter wheat grown in southwestern
998 Ontario. Can. J. Plant Sci. 95, 931-938.
999 Thioulouse, J., Chessel, D., Dole´ dec, S., Olivier, J.M., 1997. ADE-4: a multivariate analysis
1000 and graphical display software. Statistics and Computing 7, 75-83.
1001 Trail, F., 2009. For blighted waves of grain: *Fusarium graminearum* in the postgenomics era.
1002 Plant Physiol. 149, 103-110.
1003 Turmel, M.-S., Speratti, A., Baudron, F., Verhulst, N., Govaerts, B., 2015. Crop residue
1004 management and soil health: A systems analysis. Agric. Syst.134, 6-16.

1005 Vainio, E.J., Hantula, J., 2000. Direct analysis of wood-inhabiting fungi using denaturing
1006 gradient gel electrophoresis of amplified ribosomal DNA. Mycol. Res. 104, 927-936.
1007 Vanhoutte, I., Audenaert, K., De Gelder, L., 2016. Biodegradation of Mycotoxins: Tales from
1008 Known and Unexplored Worlds. Frontiers Microbiol. 7.
1009 Vanhoutte, I., De Mets, L., De Boevre, M., Uka, V., Di Mavungu, J.D., De Saeger, S., De
1010 Gelder, L., Audenaert, K., 2017. Microbial Detoxification of Deoxynivalenol (DON),
1011 Assessed via a *Lemna minor* L. Bioassay, through Biotransformation to 3-epi-DON
1012 and 3-epi-DOM-1. Toxins 9.
1013 Venkatesh, N., Keller, N.P. (2019). Mycotoxins in Conversation With Bacteria and Fungi.
1014 Frontiers Microbiol. 10.
1015 Völkl, A., Vogler, B., Schollenberger, M., Karlovsky, P., 2004. Microbial detoxification of
1016 mycotoxin deoxynivalenol. J. Basic Microbiol. 44, 147-156.
1017 Waite, I.S., O'Donnell, A.G., Harrison, A., Davies, J.T., Colvan, S.R., Ekschmitt, K., Dogan,
1018 H., Wolters, V., Bongers, T., Bongers, M., 2003. Design and evaluation of nematode
1019 18S rDNA primers for PCR and denaturing gradient gel electrophoresis (DGGE) of
1020 soil community DNA. Soil Biol. Biochem. 35, 1165-1173.
1021 Wang, L.L., Liao, Y.X., Peng, Z., Chen, L.K., Zhang, W.H., Nussler, A.K., Shi, S.J., Liu,
1022 L.G., Yang, W., 2019. Food raw materials and food production occurrences of
1023 deoxynivalenol in different regions. Trends Food Sci. & Tech. 83, 41-52.
1024 White, T.J., Bruns, T., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal
1025 ribosomal RNA genes for phylogenetics. In: Gelfand, M.A., Sninsky, D.H., White T.J.
1026 (Eds), PCR Protocols: a guide to methods and applications. Academic Press, New
1027 York, 315–322., 315-322.
1028 Wild, C.P., Gong, Y.Y., 2010. Mycotoxins and human disease: a largely ignored global health
1029 issue. Carcinogenesis 31, 71-82.

Wolfarth, F., Schrader, S., Oldenburg, E., Brunotte, J., 2016. Mycotoxin contamination and its regulation by the earthworm species *Lumbricus terrestris* in presence of other soil fauna in an agroecosystem. Plant Soil 402, 331-342.

Wolfarth, F., Schrader, S., Oldenburg, E., Weinert, J., 2013. Nematode–collembolan-interaction promotes the degradation of *Fusarium* biomass and deoxynivalenol according to soil texture. Soil Biol. Biochem. 57, 903-910.

Wolfarth, F., Schrader, S., Oldenburg, E., Weinert, J., Brunotte, J., 2011. Earthworms promote the reduction of *Fusarium* biomass and deoxynivalenol content in wheat straw under field conditions. Soil Biol. Biochem. 43, 1858-1865.

Wolfarth, F., Wedekind, S., Schrader, S., Oldenburg, E., Brunotte, J., 2015. Regulation of the mycotoxin deoxynivalenol by *Folsomia candida* (Collembola) and *Aphelenchoides saprophilus* (Nematoda) in an on-farm experiment. Pedobiologia 58, 41-47.

WRB, 2014. World Reference Base for Soil Resource 2014. International Soil Classification System for Naming Soils and Creating Legends for Soil Maps. Food and Agriculture Organization of The United Nations, Rome, Italy.

Zhou, T., He, J., Gong, J., 2008. Microbial transformation of trichothecene mycotoxins. World Mycotoxin J. 1, 23-30.

Zirbes, L., Mescher, M., Vrancken, V., Wathelet, J.-P., Verheggen, F.J., Thonart, P., Haubruge, E., 2011. Earthworms use odor cues to locate and feed on microorganisms in soil. PLoS One 6, e21927.

1058 **Table and Figure captions**

1059

1060 **Table 1:** Quantity of DON in the different treatments (SS=Straw at the Surface, SSD=
1061 SS+DON, SSE= SS+Earthworms, SSDE= SSD+Earthworms, SME= Straw Mixed with the
1062 soil+Earthworms, SMDE=SME+DON) at the different time points. ND = not determined;
1063 Traces = below the limit of quantification (LOQ = 0.0018 µg g^{-1} soil-straw mixture); 0 =
1064 below the limit of detection (LOD = 0.00034 µg g^{-1} soil-straw mixture).

1065 **Fig. 1:** Population dynamics (number of DNA copies) of A: *F. graminearum*, B: fungal
1066 community, C: bacterial community in wheat straw in the presence or absence of DON and
1067 earthworms, with straw left on the soil surface or incorporated in it. (SS=Straw at the Surface,
1068 SSD= SS+DON, SSE= SS+Earthworms, SSDE= SSD+Earthworms, SME= Straw Mixed with
1069 the soil+Earthworms, SMDE=SME+DON). The different treatments were compared at all the
1070 sampling times by analysis of variance (ANOVA) and Fisher LSD tests (p=0.05). Bars within
1071 a week with the same lower case letters are not significantly different at P <0.05. Time
1072 periods with the same upper case letters are not significantly different at P<0.05.
1073 (Soil at T0, SSE, SSDE SME, SMDE SS SSD)

1074

1075 **Fig. 2:** Impact of DON on A: culturable fungi, B: culturable bacteria, C: earthworm biomass
1076 and D: nematode densities in the microcosms with straw incorporated in the soil or placed on
1077 its surface. (SS=Straw at the Surface, SSD= SS+DON, SSE= SS+Earthworms, SSDE=
1078 SSD+Earthworms, SME= Straw Mixed with the soil+Earthworms, SMDE=SME+DON).
1079 The different treatments were compared at each sampling time by analysis of variance
1080 (ANOVA) and Fisher LSD tests (p=0.05).). Bars within a week with the same lower case

letters are not significantly different at P <0.05. Time periods with the same upper case letters are not significantly different at P<0.05.

(Soil at T0, SSE, SSDE SME, SMDE SS SSD)

Fig. 3: Principal component analysis of the T-RFLP dataset in the two-dimensional diagram from all the treatments for A: the fungal community structure, B: the bacterial community structure, C: the protozoan community structure and D: the nematode community structure Each number represents the mean of 3 biological replicates for one treatment at a specific time period. The red numbers represent the presence of DON. SS=Straw at the Surface, SSD= SS+DON, SSE= SS+Earthworms, SSDE= SSD+Earthworms, SME= Straw Mixed with the soil+Earthworms, SMDE=SME+DON.

Table 1

Treatments	Quantity of DON (DON µg g^{-1} soil-straw mixture)				
	Week 0	Week 1	Week 2	Week 8	Week 24
SME	ND	Traces	0	0	Traces
SMDE	0.831 a[†]	0.349 b	0.033 c	Traces	Traces
SSE	ND	0.105 a	0.006 b	0	0
SSDE	0.831 a	0.889 a	0.885 a	0.012 b	0
SS	ND	ND	ND	0	0
SSD	0.831 a	ND	ND	0.400 b	0

[†]Values within a column followed by the same letter are not significantly different at P≤0.05.

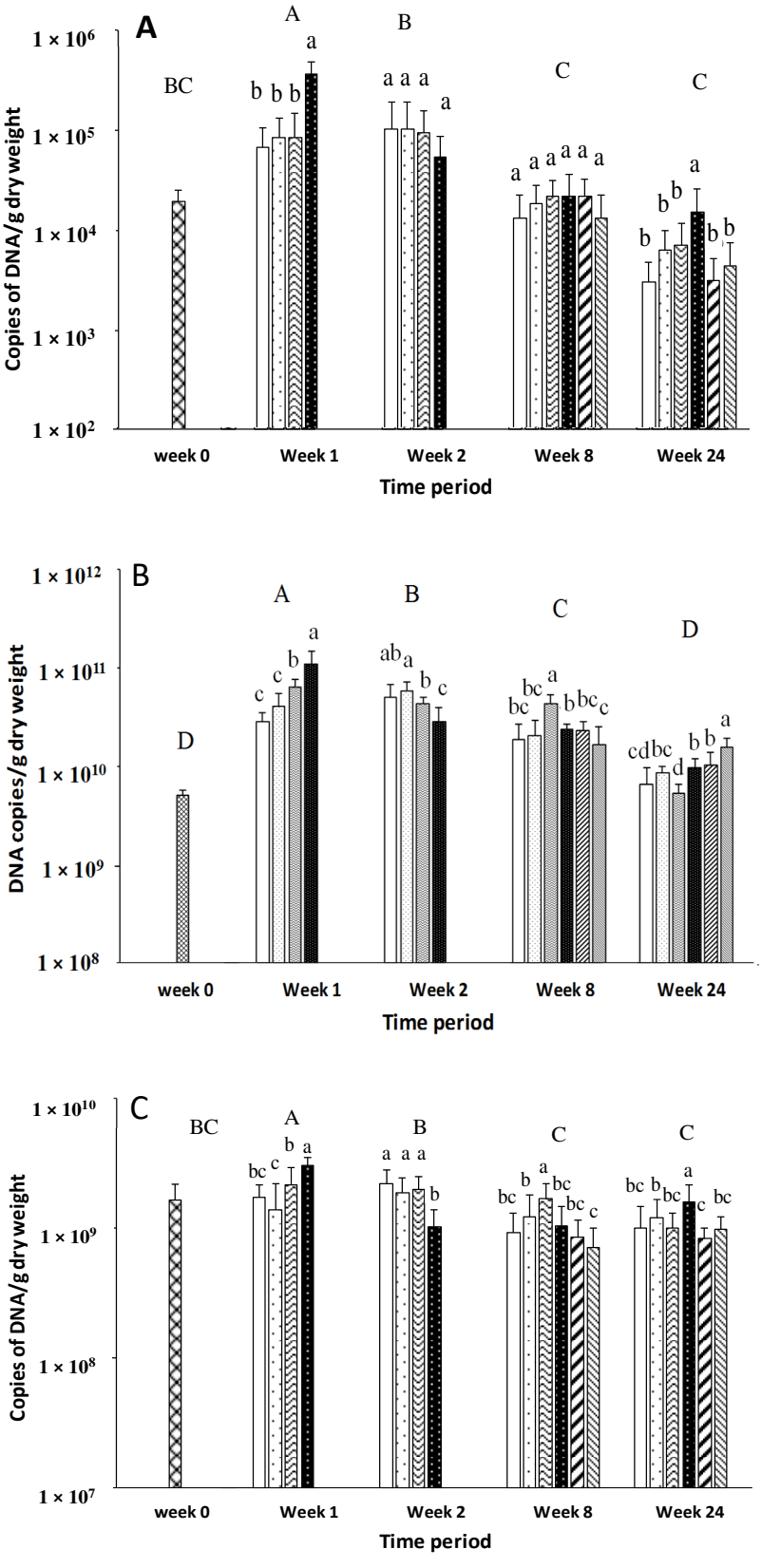

A
Copies of DNA/g dry weight
1 × 10⁶
1 × 10⁵
1 × 10⁴
1 × 10³
1 × 10²
BC
A
B
C
C
week 0
Week 1
Week 2
Week 8
Week 24
Time period
B
DNA copies/g dry weight
1 × 10¹²
1 × 10¹¹
1 × 10¹⁰
1 × 10⁹
1 × 10⁸
D
A
B
C
D
week 0
Week 1
Week 2
Week 8
Week 24
Time period
C
Copies of DNA/g dry weight
1 × 10¹⁰
1 × 10⁹
1 × 10⁸
1 × 10⁷
BC
A
B
C
C
week 0
Week 1
Week 2
Week 8
Week 24
Time period

Figure 1

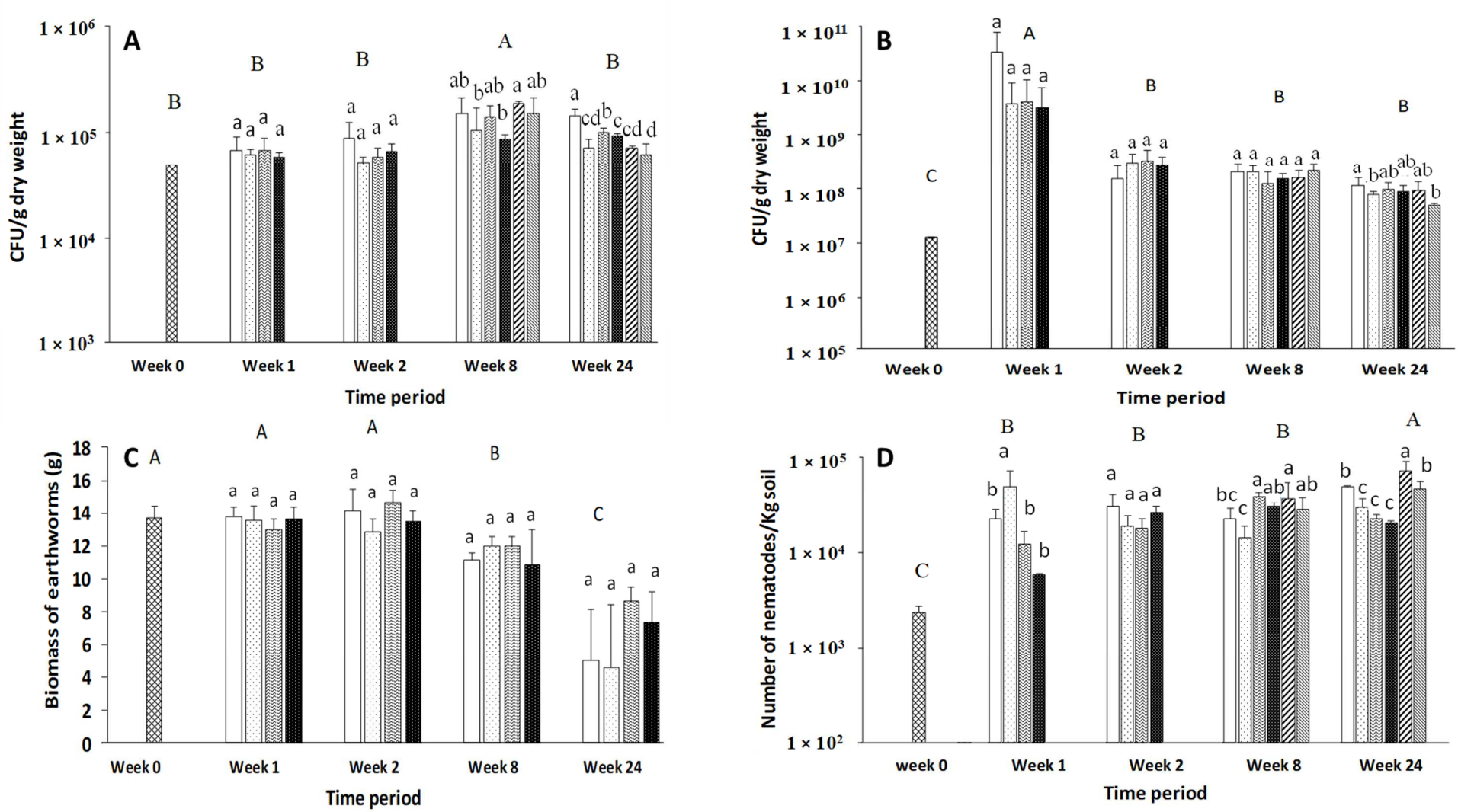
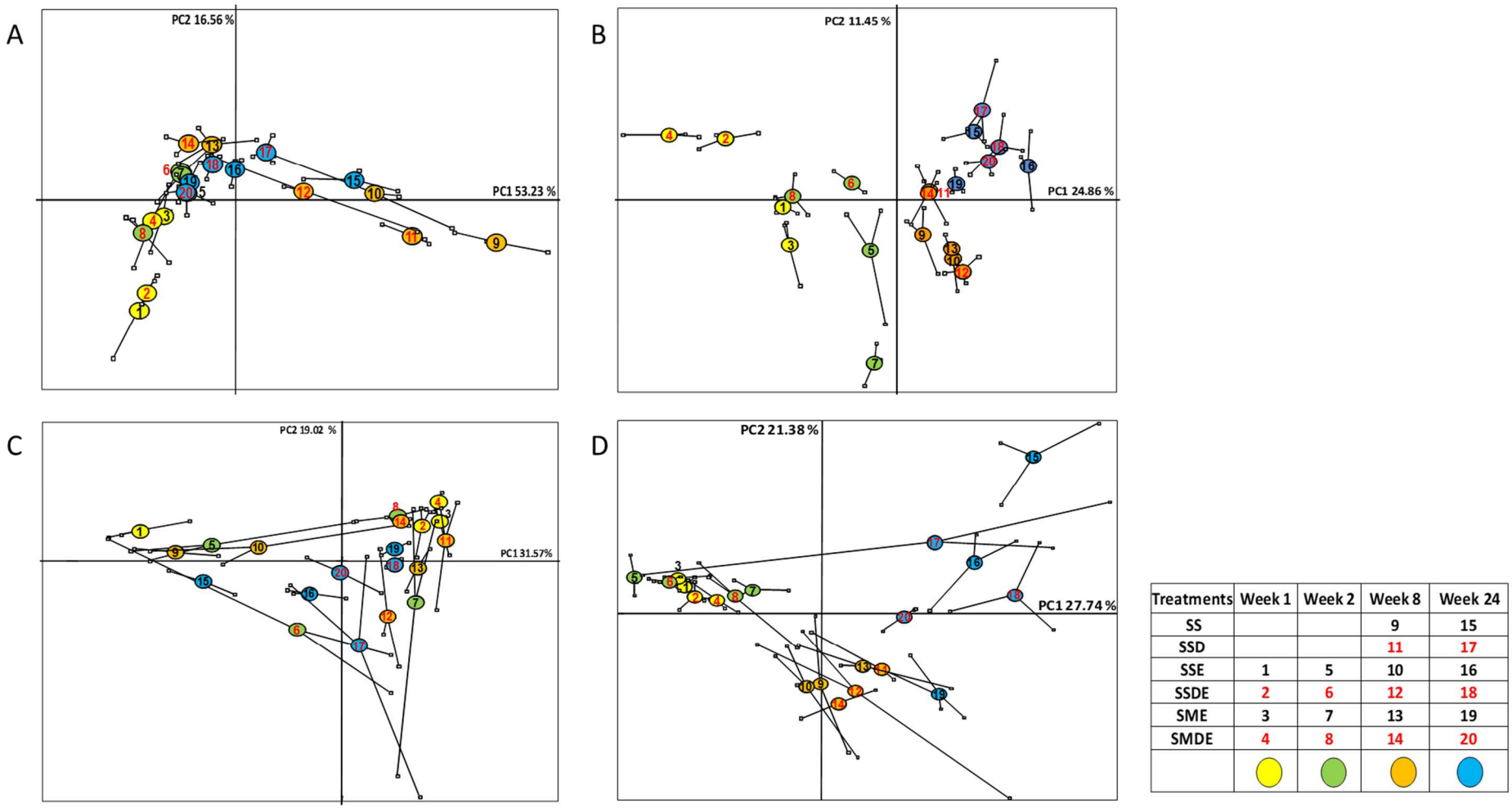

A
CFU/g dry weight
1 × 10⁶
1 × 10⁵
1 × 10⁴
1 × 10³
Week 0
Week 1
Week 2
Week 8
Week 24
Time period
B
CFU/g dry weight
1 × 10¹¹
1 × 10¹⁰
1 × 10⁹
1 × 10⁸
1 × 10⁷
1 × 10⁶
1 × 10⁵
Week 0
Week 1
Week 2
Week 8
Week 24
Time period
C
Biomass of earthworms (g)
18
16
14
12
10
8
6
4
2
0
Week 0
Week 1
Week 2
Week 8
Week 24
Time period
D
Number of nematodes/Kg soil
1 × 10⁵
1 × 10⁴
1 × 10³
1 × 10²
week 0
Week 1
Week 2
Week 8
Week 24
Time period

Figure 2



Treatments	Week 1	Week 2	Week 8	Week 24
SS			9	15
SSD			11	17
SSE	1	5	10	16
SSDE	2	6	12	18
SME	3	7	13	19
SMDE	4	8	14	20

Figure 3