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Earthworms promote the reduction of *Fusarium* biomass and deoxynivalenol content in wheat straw under field conditions

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ABSTRACT

A field experiment was conducted to elucidate ecosystem services provided by earthworms on the repression of phytopathogenic and toxinogenic fungi. The study focussed on decomposing *Fusarium culmorum*-infected and deoxynivalenol (DON)-contaminated wheat straw remaining on the soil surface as part in conservation tillage. Mesocosms were established in the topsoil of a winter wheat field located in Northern Germany, where conservation tillage has been practised for 20 years. Besides a non-earthworm treatment, two earthworm species were inoculated in the mesocosms either separately or combined: *Lumbricus terrestris* (anecic, detritivorous) and *Aporrectodea caliginosa* (endogeic, geophagous). The earthworms were exposed either to artificially *Fusarium*-infected wheat straw highly contaminated with DON or to non-infected straw serving as a control. The experiment was conducted during an eight week period after harvest from mid August to mid October. For both species, the artificially *Fusarium*-infected and DON-contaminated wheat straw was a more attractive food source than the non-infected control. In contrast to *A. caliginosa*, *L. terrestris* incorporated infected straw faster into the soil compared to control straw. Furthermore, the reduction of *Fusarium* biomass and DON concentration in wheat straw was significantly higher in the presence of *L. terrestris* than in treatments with *A. caliginosa* and without earthworms. Here, no significant differences could be measured between the *Fusarium* biomass and DON concentration in wheat straw. *A. caliginosa* seems not to be relevant for the reduction of *Fusarium* biomass and DON concentration. We concluded that amongst earthworms, anecic detritivorous species are the drivers to compensate possible negative consequences (like crop infection) of conservation tillage. They take an important role in the control of phytopathogenic and toxinogenic fungi surviving on plant residues and in the degradation of their mycotoxins.

1. Introduction

The high intensity of conventional soil tillage, namely seasonal inversion of the topsoil by ploughing, practised in traditional soil management systems, increased concerns about its negative impacts on soil structure, quality and biodiversity and on the environment (Tebrügge and Düring, 1999; Uri et al., 1999). In recent decades, the use of conservation tillage as a sustainable management measure to protect arable soils from erosion and compaction, to retain moisture and reduce production costs, has spread widely (Holland, 2004; Kassam et al., 2009; Uri et al., 1999). Using organic amendment techniques like mulching, conservation tillage promotes soil biodiversity, and enhances soil biological activity, which improves nutrient cycling and soil structural development (Hobbs, 2007; Holland, 2004).

Besides these beneficial effects of conservation tillage, a drawback may be the survival of phytopathogenic fungi like *Fusarium* species on crop residues, which may endanger the health of the following crop by increasing the infection risk for specific plant diseases (Pereyra et al., 2004; Pereyra and Dill-Macky, 2008). One of the most important soil-borne fungal diseases in cereals worldwide is *Fusarium* head blight caused by several *Fusarium* species, the

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most prevalent being *Fusarium graminearum*, *Fusarium culmorum* and *Fusarium avenaceum* (Nicholson et al., 2003; Parry et al., 1995). In wheat fields of temperate regions, *F. culmorum* is often predominant (Wagacha and Muthomi, 2007). In infected plant organs, these pathogens are able to produce mycotoxins (Parry et al., 1995). The trichothecene mycotoxin deoxynivalenol (DON) is the most frequently produced mycotoxin by *F. graminearum* and *F. culmorum* and therefore oftenly detected in cereals (Curtui et al., 2005; Pestka, 2007). Recently, Müller et al. (2010) found climatic and topographic factors to be important for DON concentrations in wheat. DON contamination of grain is an increasing problem and leads to quality losses in cereal-based feed and food and may induce toxic effects endangering the health of animals and humans (Bennett and Klich, 2003; Rotter et al., 1996). At the cellular level DON inhibits DNA, RNA and protein synthesis (Hussein and Brasel, 2001) and has adverse effects on the immune system (Smith et al., 1995). Oldenburg et al. (2007) reported higher DON concentrations in wheat grain harvested from a mulched field with conservation tillage compared to a conventionally ploughed field due to the high amount of infectious crop residues remaining on the soil surface.

To reduce the infection risk in arable land, an effective stimulation of decomposition is essential (Stemann and Lütke Entrup, 2005). A major role in acceleration of decomposition of plant material may be played by earthworms (Brown et al., 2000). *Lumbricus terrestris* and *Aporrectodea caliginosa* are keystone species of earthworms in the soil food web of temperate regions, representing two different kinds of feeding habits (Lee, 1985). The detritivorous earthworm species *L. terrestris* feeds directly on plant residues in soil systems as a primary decomposer, whereas the geophagous earthworm species *A. caliginosa* consumes pre-degraded organic matter in soil as a secondary decomposer (Lee, 1985). Nevertheless, there is clear evidence, that *Aporrectodea* species also create burrows up to the soil surface (Cook and Linden, 1996; Felten and Emmerling, 2009) and feed on soil surface litter (Ernst et al., 2009). Moody et al. (1995) found that fungal-infected wheat straw is the first food choice for earthworms compared to non-infected material. A more detailed study on the food choice revealed a clear preference of *L. terrestris* and *A. caliginosa* for *Fusarium* species (Bonkowski et al., 2000). Recently, laboratory experiments by Oldenburg et al. (2008) and Schrader et al. (2009) gave evidence that *L. terrestris* takes part in the efficient degradation of both *Fusarium* biomass and DON occurring in crop residues. However, a validation of these earthworm mediated ecosystem services under field conditions has not been carried out so far. Furthermore, the role of other earthworm species belonging to a different functional group than *L. terrestris* and the functional interaction of ecological groups with respect to these ecosystem services has not been studied.

Therefore, we conducted a field study on plant pathogen repression and mycotoxin degradation as ecosystem services provided by earthworms of two different functional groups. We hypothesized that earthworms (*L. terrestris*, *A. caliginosa*) significantly reduce the biomass of the soil-borne phytopathogenic fungus *F. culmorum* and the content of its mycotoxin deoxynivalenol (DON) in infected wheat straw remaining on the soil surface as part of conservation tillage. We assumed that, based on their different ecological classification, the anecic, detritivorous primary decomposer *L. terrestris* provides pre-digested plant material as food for the endogeic, geophagous secondary decomposer *A. caliginosa* by burial activity, which might enhance the degradation of *Fusarium* biomass and DON concentration in straw residues by species interaction. For this purpose, the earthworm species *L. terrestris* and *A. caliginosa* were fed on artificially *Fusarium*-infected and DON-contaminated wheat straw in a mesocosm study under field conditions. Both species are very common in arable soil and widespread in temperate regions (Lee, 1985). In order to contribute to the understanding of the functional role of earthworms in agroecosystems, we asked the following questions: (1) Do earthworms play an important role in controlling plant pathogen fungi and in degrading their mycotoxins? (2) Does the interaction of two earthworm species of two different functional groups enhance the efficiency of one single species?

2. Materials and methods

2.1. Environmental conditions and site description

The experimental field was located at Adenstedt near Hildesheim in the southwest of Braunschweig, Northern Germany (9°56′E 52°00′N, 196 m a.s.l.). The climate conditions of this region are characterised by a mean annual air temperature of 8 °C and an average precipitation rate of 700 mm y^{-1}. Fig. 1 shows the actual air

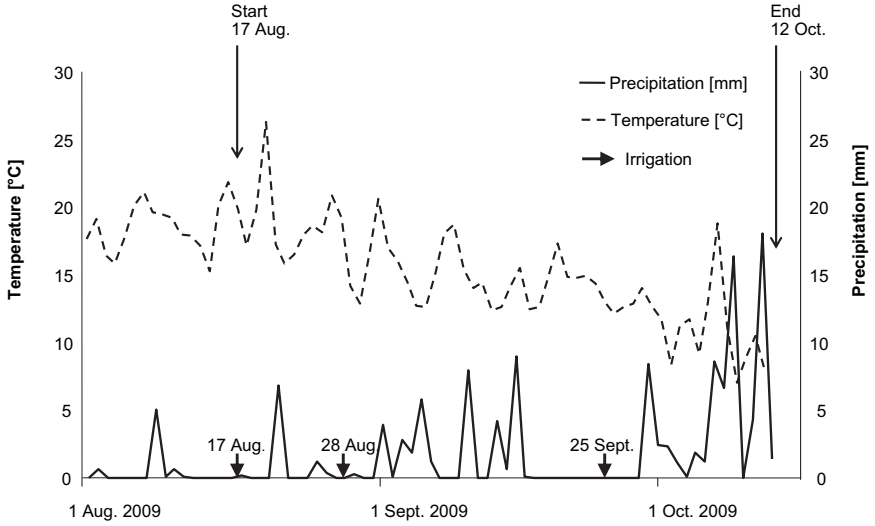

Fig. 1. Mean daily temperature and precipitation at the experimental field in Adenstedt (Northern Germany) from 1 August to 12 October 2009 (data provided by the Institute of Physical Geography and Landscape Ecology, University of Hannover). Dates of start (17 August) and end (12 October) of the field experiment as well as the three dates of manual irrigation.

temperature and seasonal precipitation for about two weeks before and during the field experiment. The data were provided by the Institute of Physical Geography and Landscape Ecology, University of Hannover, which maintains a meteorological station at that site.

The experiment was established in 2009 in a winter wheat field that was harvested two weeks previous to the study and where conservation tillage had been practised for 20 years. The experimental area within the field was 18 m wide and 40 m long. The soil was a Luvisol derived from loess as parent material with a pH value of 7.3 and a mean organic matter content of 2.1%. The soil texture is characterised by 12% clay, 85% silt and 3% sand resulting in a silt loam. At the beginning of the experiment, the relative water holding capacity (WHC) of the soil was 58.7%.

2.2. Soil, straw and earthworms

The experiments were conducted with topsoil from the agricultural field described above. The soil was stored at 4 °C until further treatment. Seven days before filling the mesocosms, the soil was defaunated by freezing at −20 °C for 24 h and thereafter thawed at room temperature for 24 h. This freezing–thawing cycle was repeated three times, which significantly reduces the number of soil microarthropods and annelids (Huhta et al., 1989). The soil was macroscopically cleared of organic plant residues like straw or roots and sieved (mesh size 2 mm).

Winter wheat (*Triticum aestivum cv.* Tommi) was cultivated at an experimental site of the University of Göttingen (Germany). During flowering, wheat plots of 4 m^2 in size had been sprayed with 400 ml fungal spore suspension made of three strains of *F. culmorum* mixed in using the wetting agent Tween 20 (0.5 ml l^{-1}). The conidiospore concentration of the suspension was 3×10^5 ml^{-1}. For more details see Oldenburg et al. (2008) and Schrader et al. (2009). In infected plant organs *F. culmorum* produced its mycotoxin DON, which reached a level of 123,070 ± 5886 μg kg^{-1} when the plant material was collected. Chopped straw of approximately 1.0 cm length was used for the experiments. This length was convenient to simulate a mulch layer with straw in close contact to the soil on an experimental surface area of about 113 cm^2 in each mesocosm. Winter wheat straw, which was not artificially infected with *Fusarium* served as a control (in the following called "non-infected") and contained DON at a low level of 659 ± 103 μg kg^{-1}.

Adult individuals of the detritivorous earthworm species *L. terrestris* and adult and subadult individuals of the geophagous species *A. caliginosa* were adapted to the soil conditions for 10 days by keeping them in plastic jars containing the soil described above, covered with wheat straw of the control at 15 °C (±1 °C). *L. terrestris* was purchased from a commercial supplier whereas *A. caliginosa* was collected from the experimental field described above. Before they were placed into the experimental mesocosms, the earthworms were washed with water to remove adhesive residues and mucus.

2.3. Experimental design

The experimental units (mesocosms) were cylinder-shaped bags out of nylon-gauze of 12 cm × 40 cm in size (diameter × height). A mesh size of 20 μm was chosen to enable an exchange of air and water with the surrounding soil in the field but prevent other soil fauna from immigrating. Four days prior to earthworm inoculation, 4.7 kg of soil (wet weight) moistened to 18% w/w were filled in each mesocosm resulting in a 25 cm high soil column with a bulk density of 1.35 g cm^{-3}, which represented real field conditions. The C_{org} content of soil was 1.2%. Portions of 8 g air-dried wheat straw per mesocosm were moistened by spraying with water and added as a layer in close contact with the soil surface. In total, there were 48 mesocosms. One set of 24 mesocosms was applied with *Fusarium*-infected straw and another set (24 mesocosms) with non-infected straw. Each set was divided into 4 treatments with six replicates for each treatment: one treatment with *L. terrestris* (2 individuals), one treatment with *A. caliginosa* (6 individuals), one mixed treatment with *L. terrestris* (1 individual) and *A. caliginosa* (3 individuals) and one treatment without earthworms as control. Additional small quantities of soil and straw (infected and non-infected straw) were retained and stored at −20 °C until further processing for detecting the initial DON and FPE concentrations (see Sections 2.6 and 2.7). The mesocosms were supplied with approximately the same earthworm biomass per treatment on average. The average weight for the single species treatment with *L. terrestris* was 9.1 ± 1.1 g, for single species treatment containing *A. caliginosa* the average biomass was 1.6 ± 0.2 g. For the mixed species treatment the average biomass was 5.6 ± 1.8 g. After the introduction of the earthworms, each mesocosm was closed with a clip.

Preparing the establishment of the experiment in the field, 48 pits were drilled in the ground 30 cm deep with a hydraulic soil driller. The pits were placed in three lines of 30 m in length. The interval between each pit was 2 m whereas the distance between each line was 7 m and 3 m, respectively, to avoid drilling in wheeling tracks. The mesocosms were randomly introduced into the prepared pits in close contact to the surrounding soil. The experiment was carried out during a period of 8 weeks from mid August to mid October (Fig. 1). Due to a temporary lack of precipitation, all 48 mesocosm locations (a 1 m^2 area with a mesocosm in the centre, respectively) were manually irrigated (10 mm) at three dates (Fig. 1) to maintain soil moisture.

2.4. Determination of soil surface cover

The area of the soil surface was determined by scanning top view photographs of the mesocosms at the start and, after 8 weeks of field exposure, at the end of the experiment. The specific areas of uncovered soil and those covered with straw were evaluated with the colour analysis program WinRHIZO 2002 (Régent Instruments Inc.).

2.5. Sampling and sample processing

Soil samples ($n = 8$; 0–30 cm soil depth) were randomly taken from the experimental field at the beginning and the end of the experiment to measure the soil water content. The soil moisture was gravimetrically determined after drying at 105 °C for 24 h.

At the end of the experiment, the mesocosms were excavated from the field and transported to the laboratory. The remaining straw on the soil surface was removed from the mesocosms. During straw sampling, great care was taken to mechanically separate adhesive soil, but washing was avoided to prevent elution of DON, which is water soluble. Furthermore, earthworm casts were collected. Soil samples were taken from areas that visually appeared to be unaffected by earthworm activity. Finally, the earthworms were removed, carefully cleaned off soil particles and weighed individually to determine their biomass for comparison with their initial biomass. All samples were then stored at −20 °C in readiness for analytical preparation. Part of the soil samples were used for gravimetrical determination of soil moisture conditions within the mesocosms. The soil samples were dried at 105 °C for 24 h.

All samples, including the parent materials at the start of the experiment, were dried by lyophilisation for 24 h. Straw was ground using a mixer mill (MM 400, Retsch GmbH, Haan,

Germany). Samples of soil and casts were manually homogenized with a mortar to obtain a fine powder (<0.5 mm).

2.6. Determination of Fusarium protein equivalents

As a measure of *Fusarium* biomass, *Fusarium* protein equivalents (FPE) were quantified with a double antibody sandwich (DAS) ELISA by using *Fusarium*-specific antibodies and protein standards, according to the procedure described by Oldenburg et al. (2008). Samples of 0.5 g of wheat straw and 1.0 g of soil or earthworm casts were taken for the assay. The limits of quantification were 78 μg FPE kg^{-1} for all samples.

2.7. Determination of deoxynivalenol

The DON concentrations were quantified by using a competitive ELISA (ELISA test kit 'Ridascreen DON', product no. 5906 from R-Biopharm, Darmstadt, Germany), according to the procedure described by Oldenburg et al. (2008). The initial sample weight was 1.0 g for wheat straw and 2.5 g for soil and earthworm casts. The limits of quantification were 37 μg kg^{-1} for soil, casts and non-infected straw and 74 μg DON kg^{-1} for infected wheat straw.

2.8. Statistics

The Kolmogorov–Smirnov test confirmed that all data were normally distributed. For this reason, a repeated measures analysis of variance (RM-ANOVA) was carried out to compare treatment effects of earthworm species (*L. terrestris*, *A. caliginosa*, Mix), *Fusarium* treatment (infected, non-infected) and date (start, end of the experiment). A posteriori test (post-hoc test) was implemented for determine differences among the means. According to Day and Quinn (1989) we chose a Tukey's HSD (honestly significant difference) test for pairwise multiple comparisons within the factor "earthworm species". All statistical analyses were done using the software package SPSS for Windows version 13.

3. Results

3.1. Soil moisture conditions

The average soil water content of the experimental field increased significantly from an initial content of 16.1% (relative water holding capacity 58.7%) to a final content of 22.7% (WHC 82.8%). A similar development was found for the moisture in the mesocosms. Their initial average moisture was 18.2% (WHC 66.4%). At the end of the field experiment the soil water content was significantly higher in all mesocosms, but no significant differences could be determined between the treatments. The moisture in the mesocosms varied between 19.8% (WHC 72.2%) and 20.2% (WHC 73.7%).

3.2. Earthworm biomass

At the end of the experiment a loss of 4 out of 36 individuals of *L. terrestris* and 27 out of 108 individuals of *A. caliginosa* was recorded. That is a recapture rate of 89% and 75% for *L. terrestris* and *A. caliginosa*, respectively. The mean individual biomass of the remaining earthworms was determined for each mesocosm and compared with the respective mean initial individual body weight. According to RM-ANOVA, there was no statistically significant difference between the treatments (infected *versus* non-infected straw) in the change of earthworm biomass ($F = 0.179$; $P = 0.678$).

3.3. Soil surface cover

Statistically significant effects on the incorporation of straw were revealed for all main effects and two of their interactions (earthworm species × date; *Fusarium* treatment × date) (Table 1).

In the presence of earthworms, the surface area of the soil covered by either non-infected or infected straw decreased significantly during the experiment (Fig. 2). For the mesocosms containing *L. terrestris*, the infected wheat straw was incorporated more efficiently into the soil, as the soil surface cover was reduced more (−21%) compared with non-infected straw (−13%). A similar development was observed for the mixed treatments, where 12% of the infected straw was incorporated while the reduction of non-infected straw was 8%. The soil surface cover with infected wheat straw of the mesocosms containing *A. caliginosa* was reduced by 1.4% whereas in case of non-infected straw the soil surface cover was reduced by 0.8%. There was no reduction of surface straw coverage in treatments without earthworms hence the soil surface cover remained 100%.

3.4. Concentrations of FPE in wheat straw

In aboveground straw, significant effects were recorded for all three factors and their interactions, but in case of belowground straw only the factors "*Fusarium* treatment" and "date" and their interaction had a significant influence (Table 2).

The initial *Fusarium* biomass in the infected straw on the soil surface was 40,971 ± 1617 μg kg^{-1}. After 8 weeks of field exposure the FPE concentration of all earthworm treatments decreased (Fig. 3). The *Fusarium* biomass was degraded by 75% in control mesocosms without earthworms. In treatments containing *L. terrestris* or both earthworm species, the FPE concentration was reduced by 98%, which is equivalent to one order of magnitude. In mesocosms with *A. caliginosa* the degradation of the *Fusarium* biomass was 74%.

The *Fusarium* biomass initially present in non-infected aboveground straw of 1176 ± 150 μg kg^{-1} was reduced in all treatments, but a significant degradation of 54% could only be measured in treatments without earthworms. In treatments containing *L. terrestris* and *A. caliginosa* the reduction was 26% and 8%, respectively, whereas the FPE concentration of the mixed mesocosms was reduced by 25%.

Regarding the FPE contents of infected straw the earthworms incorporated into the soil, a mean reduction of about one order of magnitude was determined (Fig. 3). This reduction was 95% in case of mixed treatments and those containing *L. terrestris*. The *Fusarium* biomass in belowground straw in mesocosms with *A. caliginosa* was reduced by 96% which differed significantly from treatments containing *L. terrestris*.

Table 1
The soil surface straw cover. *F*-values of RM-ANOVA on the effects of earthworm species (*L. terrestris, A. caliginosa*, Mix), *Fusarium* treatment (*Fusarium*-infected wheat straw, non-infected) and date (start, end of the experiment).

	Soil surface cover
Earthworm species (E)	12.06**
Fusarium treatment (F)	9.40*
Date (D)	50.53***
F × E	2.55
E × D	12.06**
F × D	9.40*
F × E × D	2.55

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

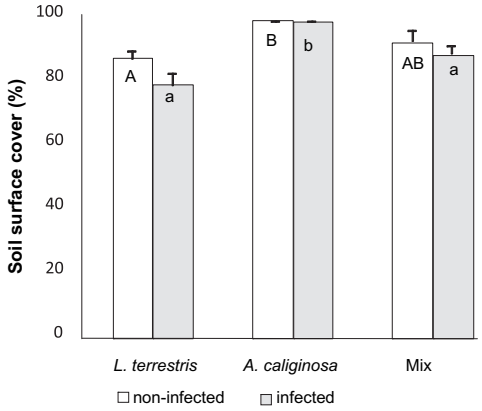

Fig. 2. Mean (+SE) relative soil surface cover with straw residues in mesocosms with *L. terrestris*, *A. caliginosa* and both earthworm species (Mix) separated in *Fusarium*-infected and non-infected treatments after an experimental period of 8 weeks. Different letters indicate bars to be significantly different ($P < 0.05$) according to Tukey's HSD test; small letters refer to the infected and capital letters to the non-infected treatment.

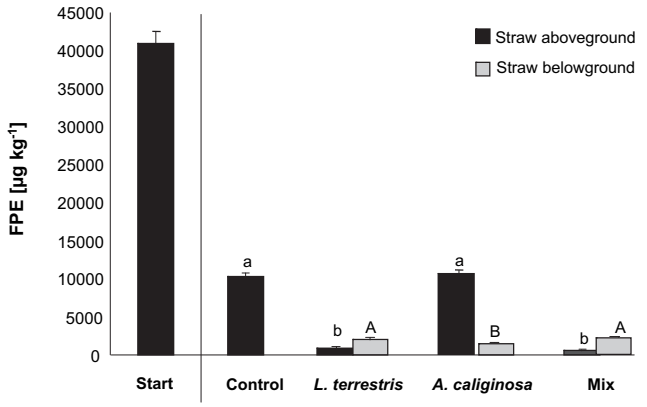

Fig. 3. Mean (+SE) concentrations of FPE (*Fusarium* Protein Equivalents) in infected winter wheat straw above- and belowground in field soil mesocosms inoculated with the earthworm species *L. terrestris*, *A. caliginosa*, both species (Mix) or without earthworms (control) at the beginning (start) and the end (8 weeks later) of the field experiment. Different letters indicate statistically significant difference in means (Tukey's HSD test; $P < 0.05$); small letters refer to straw aboveground and capital letters to straw belowground. The start concentration served as reference value but was not included in Tukey's test.

3.5. Concentrations of FPE in soil and casts

At the start of the experiment the initial FPE concentration in soil was 315 ± 48 µg kg^{-1}. A reduction could be measured for all infected treatments, especially for those without earthworms. A similar development of *Fusarium* biomass in soil was observed for the non-infected treatments. RM-ANOVA on soil FPE concentrations revealed no significant influence for the factor "earthworm species" whereas for "date" and the interaction of "date" and "earthworm species" a statistically significant impact was calculated (Table 2). Furthermore, the interaction of all three factors significantly influenced the FPE concentration in soil (Table 2).

The FPE content in soil of the infected treatments containing earthworms (*L. terrestris, A. caliginosa* and mixed mesocosms) did not change significantly. For the infected treatments without earthworms a reduction of *Fusarium* biomass concentration in soil was determined.

For the non-infected treatment without earthworms a reduction of FPE concentration in soil was determined. The FPE content in mesocosms containing earthworms (*L. terrestris* and *A. caliginosa* in single species treatment as well as in mixed treatments) did not alter significantly.

Fusarium biomass detected in earthworm casts of all treatment did not differ significantly compared to the FPE content of the soil at the beginning of the experiment.

3.6. Concentrations of DON in wheat straw

The impact of the studied factors on the DON concentration of the remaining aboveground wheat straw is presented in Table 3. Significant effects were recorded for "*Fusarium* treatment" and "date" as well as their interaction.

In the infected aboveground wheat straw, an initial DON concentration of 123,070 ± 5886 µg kg^{-1} was determined (Fig. 4). After the time of field exposure the DON concentration of the treatment without earthworms was reduced by 94%, which is equivalent to one order of magnitude. For both treatments, mesocosms containing *L. terrestris* and both earthworm species, a degradation of DON concentration of two orders of magnitude (99%) was observed. The DON concentration in the treatment with *A. caliginosa* was reduced also by 92%.

Regarding the DON concentrations of infected straw the earthworms incorporated into the soil, a mean reduction of two orders of magnitude (99%) was determined for all treatments (Fig. 4). No significant differences were detected between the different DON concentrations in the incorporated straw of the three earthworm treatments.

The DON concentration in the non-infected straw aboveground of initially 659 ± 193 µg kg^{-1} decreased in all treatments. In mesocosms without earthworms the reduction was 84%. For the treatment containing *L. terrestris*, a degradation of 95% was determined. However, only two out of six mesocosms for the *L. terrestris* treatment contained positive DON concentrations. For those containing *A. caliginosa*, the reduction was 91%. No DON could be detected in the non-infected straw of the mixed mesocosms (<37 µg kg^{-1}). The DON concentration for non-infected belowground straw was below quantification limit (<37 µg kg^{-1}) in all treatments.

Table 2
The FPE concentration in wheat straw above- and belowground and in soil. *F*-values of RM-ANOVA on the effects of earthworm species (*L. terrestris*, *A. caliginosa*, Mix), *Fusarium* treatment (*Fusarium*-infected wheat straw, non-infected) and date (start, end of the experiment).

	Straw aboveground	Straw belowground	Soil
Earthworm species (E)	14.39***	0.02	1.97
Fusarium treatment (F)	4483.98***	2128.81***	0.66
Date (D)	2469.78***	1755.06***	6.29*
F × E	18.16 ***	0.39	8.53**
E × D	15.19***	0.01	1.97
F × D	2867.31***	3720.88***	0.66
F × E × D	18.76***	0.70	8.53**

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Table 3
The DON concentration in wheat straw aboveground. *F*-values of RM-ANOVA on the effects of earthworm species (*L. terrestris*, *A. caliginosa*, Mix), *Fusarium* treatment (*Fusarium*-infected wheat straw, non-infected) and date (start, end of the experiment).

	Straw aboveground
Earthworm species (E)	1.21
Fusarium treatment (F)	2383.85***
Date (D)	1237.74***
F × E	1.21
E × D	0.78
F × D	1247.83***
F × E × D	0.79

***$P < 0.001$.

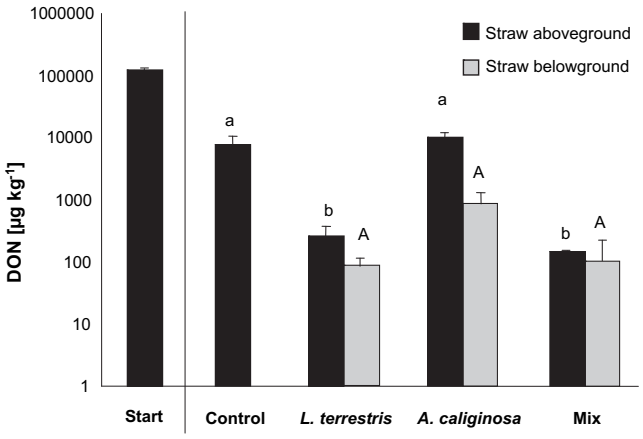

Fig. 4. Logarithmic presentation of mean (+SE) concentrations of DON (deoxynivalenol) in infected winter wheat straw above- and belowground in field soil mesocosms inoculated with the earthworm species *L. terrestris*, *A. caliginosa*, both species (Mix) or without earthworms (control) at the beginning (start) and the end (8 weeks later) of the field experiment. Different letters indicate statistically significant difference in means (Tukey's HSD; $P < 0.05$); small letters refer to straw aboveground and capital letters to straw belowground. The start concentration served as reference value but was not included in Tukey's test.

3.7. Concentrations of DON in soil and casts

Neither in the undigested soil nor in earthworm casts positive DON concentrations (<37 µg kg^{-1}) could be detected at the start and after 8 weeks of field exposure.

4. Discussion

L. terrestris promoted the degradation of *Fusarium* biomass and DON in wheat straw, because the FPE content as well as the DON concentration decreased more efficiently than in its absence or in the presence of *A. caliginosa*. This finding is in line with results of Oldenburg et al. (2008) and Schrader et al. (2009), who also measured a significant decline of DON and FPE in wheat straw in the presence of *L. terrestris* in laboratory studies. Both authors suggest metabolic interactions between soil microorganisms and earthworms. A notable behaviour of anecic earthworms concerning litter decomposition is the ploughing-in effect described by Cortez (1998) and Cortez and Bouché (1998), which initiates and promotes microbial litter decomposition. Anecic earthworm species, like *L. terrestris*, create structures at the soil surface known as "earthworm middens" by casting activities and litter collection (Brown et al., 2000; Subler and Kirsch, 1998). Providing improved conditions of microclimate and a higher diversity of habitat structure for soil organisms (Schrader and Seibel, 2001), middens are hotspots of increased abundance of soil micro- and mesofauna as well as enhanced microbial activity and nutrient availability (Subler and Kirsch, 1998). According to Doube and Brown (1998), the consequences of the interactions between earthworms and microbial communities vary with the ecological category to which the earthworms belong. Also, the present study gave clear evidence that the impact of the introduced earthworms on the degradation of *Fusarium* biomass and DON concentration in this investigation also depends on their ecological group. According to our results, the role of the endogeic geophagous species *A. caliginosa* seemed to be minor. The interaction between *A. caliginosa* and the anecic detritivorous species *L. terrestris* did not enhance the degradation of FPE and DON compared to the *L. terrestris* single species treatment. Compared with *A. caliginosa*, the influence of *L. terrestris* seemed to be crucial. The primary decomposer of surface litter, *L. terrestris*, can be suggested as the driver of the degradation process.

Relating the decline of *Fusarium* biomass and DON concentrations in remaining straw to earthworm activity, it is likely that the earthworms induced a priming effect (Binet et al., 1998; Brown, 1995) by secretion of cutaneous mucus containing highly bioavailable compounds enhancing microbial activity (Brown, 1995; Schrader et al., 2008). According to Kuzyakov (2010), the priming effect is an initiation of increased intensity of dead organic matter turnover induced by pulses or continuous input of fresh organics, e.g., in the rhizosphere, detritusphere or drilosphere. Cutaneous mucus of earthworms is rich in, e.g., polysaccharides (Zhang and Schrader, 1993), which might have created hotspots of microbial activity in above- and belowground straw within the mesocosms. Besides direct feeding, inducing a priming effect seems to be a second important factor of earthworm activity which promotes the reduction of *Fusarium* biomass and DON concentration.

The reduction of *Fusarium* biomass in the non-infected treatments was much lower than in treatments containing infected straw. It can be assumed that non-infected straw was less preferred food for earthworms, possibly because of its more un-decomposed state and recalcitrant compounds cellulose, hemicelluloses and lignin (Bowen and Harper, 1990; Harper and Lynch, 1981). Consequently, the incorporation of the non-infected straw was lower. However, it remains an open question why the percentage degradation of *Fusarium* biomass in the control treatment without earthworms was higher compared to the earthworm treatments.

The highly *Fusarium*-infected wheat straw seemed to be more attractive to the earthworms, especially to *L. terrestris*, than the non-infected straw, because decomposition was faster in mesocosms where the infected straw was offered. Similar results have been reported by Oldenburg et al. (2008). In contrast to *L. terrestris*, *A. caliginosa* appeared not to feed on intact wheat litter but rather on organic matter that had previously been partly decomposed. Similar results were reported for the endogeic species *Octolasion lacteum* by Bonkowski et al. (1998). Presumably, the straw provided may have been insufficiently pre-degraded to fulfil the requirements of *A. caliginosa* as a secondary decomposer. Generally, it is well documented that earthworms do not feed at random (Bonkowski et al., 2000). Studies on food selection with *L. terrestris* and *A. caliginosa* have shown distinct preferences for *Fusarium* spp. (Bonkowski et al., 2000; Cooke, 1983; Moody et al., 1995). Furthermore, soil fungi are regarded as a primary food source for earthworms (Brown, 1995). However, Edwards and Fletcher (1988) reported an inhibition of earthworm growth and even lethal effects on earthworms due to mycotoxin producing fungi. These findings are contradictory to the results of this study, although some individuals were lost due to unknown reasons, which were not related to specific treatments. Specific mesocosm effects could be excluded, as the soil moisture conditions in the field soil and the mesocosms developed similarly during the experiment. No significant differences in soil moisture between the single mesocosms were observed, indicating equal experimental conditions within the treatments.

In general, soils managed by conservation tillage are regarded to have a higher biodiversity, as well as higher earthworm numbers, compared to conventionally tilled soils (Edwards et al., 1995; Holland, 2004). Furthermore, it is documented that earthworm populations and community structure were affected directly by the tillage system used (Edwards and Lofty, 1982; Langmaack, 1999). Edwards and Lofty (1982) for example, observed an increase of earthworm populations up to 30% after 8 years of conservation tillage. Especially deep burrowing earthworm species like *L. terrestris* benefit from reduced tillage (Edwards, 1983; Edwards et al., 1995; Joschko and Rogasik, 2002).

Considering the described drawback of conservation tillage, i.e., the survival of phytopathogenic fungi, Pereyra et al. (2004) found

a significant relationship between decomposition of wheat residues and the recovery of *Gibberella zeae*, the sexual anamorph of *F. graminearum*. Furthermore, they could prove that *F. graminearum* declined faster in buried plant material than in residues on the soil surface. The infected surface residue is the most important infection source, endangering the aboveground organs of the following crop during the whole vegetation period. When infected residues are buried into the topsoil by earthworms, the possibility that remaining straw is still infectious for seedlings of the following crop is efficiently restricted, as the decomposition of plant material is accelerated. For agroecosystems, Whalen and Parmelee (1999) estimated organic matter consumption by *L. terrestris* at 8.1–10.6 Mg ha^{-1} y^{-1} and by *Aporrectodea tuberculata* (endogeic species) at 3.6–6.6 Mg ha^{-1} y^{-1}. To optimize this important turnover of organic material, agrotechnical residue treatments like chopping would be advantageous to facilitate the degradation of infectious plant residues by earthworms. Just recently, Vogelgsang et al. (2011) reported from their on-farm experiments that small *versus* large and spliced *versus* intact residue pieces being more favourable for competing saprophytes and potential antagonists of *Fusarium* due to a larger surface and higher humidity. By this means, earthworms play an important role in reducing infection risk of plant pathogen fungi like *Fusarium* species.

5. Conclusions

According to the results of the present investigation anecic detritivorous species are the drivers to compensate for the enhanced risk of fungal crop diseases deriving from conservation tillage practises. As in particular *L. terrestris* promoted the reduction of *Fusarium* biomass content and DON concentration in wheat straw, it can be concluded that earthworms play an important role in plant pathogen repression and mycotoxin degradation as ecosystem services. The interaction with endogeic geophagous species seems not to enhance the efficiency of anecic detritivorous species regarding these services.

The sustainable delivery of ecosystem services by earthworms, as shown in the present study, promotes soil health in arable fields under conservation tillage combined with mulching. Besides cultivation of less susceptible cultivars and chemical plant protection measures, residue treatment by mulching machinery is an appropriate method to reduce the risk of crop infection with *Fusarium*. Therefore, the farmer's technical residue management promotes natural mechanisms of self-regulation in the soil system and maintains food and feed quality.

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