**Lumbricus terrestris** regulating the ecosystem service/disservice balance in maize (*Zea mays*) cultivation

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Abstract

**Background and aim** Plant pathogenic and mycotoxin-producing *Fusarium* species are globally widespread and lead to large annual yield losses in maize production (ecosystem disservice). Systems with reduced tillage and mulching are particularly under threat. In the present study, the bioregulatory performance (ecosystem service) of the common earthworm species *Lumbricus terrestris* was analysed regarding the suppression of three economically relevant *Fusarium* species, and the reduction of their mycotoxins in the maize mulch layer, taking into account the size of maize residues.

**Methods** A mesocosm field experiment was conducted in a reduced tillage long-term field trial on loam soil. Artificially *Fusarium*-infected maize residues of two size classes were used as a mulch layer. Impacts of the earthworm species on DNA amounts of *Fusarium graminearum*, *F. culmorum*, and *F. verticillioides* and concentrations of the mycotoxins deoxynivalenol (DON), 3-acetyldeoxynivalenol (3-AcDON), and zearalenone (ZEN) were analysed.

**Results** The results reflect that *Fusarium* regulation by *L. terrestris* was species-specific and covered the whole spectrum from suppression (*F. graminearum*) to slight promotion (*F. verticillioides*). Regarding the mycotoxins, a significant acceleration of the degradation of all three toxins was detected. Fine chopping of the chaff (< 2 cm) did not significantly alter the earthworms’ regulatory capacity.

**Conclusion** While *L. terrestris* can shift the ecosystem service/disservice balance in both directions with respect to *Fusarium* regulation, it shifts it towards ecosystem services with respect to mycotoxin degradation. In synergy with adapted agricultural management, this natural bottom-up effect can help to keep soils healthy for sustainable production in the long run.

**Keywords** *Fusarium* • Earthworms • Bioregulation • Pathogen suppression • Mycotoxin degradation

Introduction

*Fusarium* species are omnipresent in soils and on plants (Wenda-Piesik et al. 2017) and, as plant pathogenic fungi of cereals, play an essential economic role worldwide (Ferrigo et al. 2016). While in temperate latitudes especially the species *Fusarium graminearum* and *Fusarium culmorum* led to high yield losses in the past (Bottalico and Perrone 2002; Leplat et al. 2013), the distribution of *Fusarium verticillioides* (formerly...
Fusarium moniliforme), as most common species in maize, was limited to warmer regions (Aguin et al. 2014; Bottalico 1998). However, due to rising temperatures in the context of climatic change, this species has increasingly spread even in originally colder, humid regions in recent years (Czembor et al. 2015; Pfördt et al. 2020). As the temperature rise continues and a further increase in maize cultivation is predicted (EC (European Commission) 2018; Pavlik et al. 2019), an increase in infestation rates by F. verticillioides in temperate latitudes is expected in the near future (Oldenburg et al. 2018).

Farmers in these regions are therefore confronted with the risk of infestation by a rising number of Fusarium species and face the major challenge of preventing and effectively controlling infections to keep their plants and soils healthy and to ensure sustainable yields. In this context, two aspects of infestation must be taken into account, in both, prevention and control measures. First, an infestation leads to various plant disease patterns such as Fusarium head blight, ear rot, or stem rot, usually associated with reduced crop yields (Ferrigio et al. 2016). Second, many Fusarium species can produce toxic metabolic products such as trichotecenes, fumonisins, or zearalenone. These mycotoxins pose a health risk to humans and animals (Ferrigio et al. 2009; Tissier et al. 2016); the lack of fungicides (Masiello et al. 2019; Wegulo et al. 2015) hamper the efforts to combat F. verticillioides infestation and prevent its spread. Linked to maize cultivation’s economic value as essential crop production, its predicted increase in upcoming years (EC (European Commission) 2018), and the increasing implementation of reduced tillage as a contribution to sustainable production intensification (Kassam et al. 2009) also in maize cultivation systems (Claassen et al. 2018), it becomes apparent that Fusarium infections currently are, and in particular will be a challenge for securing high-quality yields, now and in the future.

Against this background and considering that soils treated by reduced tillage usually show higher functional soil biodiversity than ploughed soils (Pelosi et al. 2014), soil self-regulation and intrinsic biocontrol mechanisms as natural bottom-up effects have increasingly come into the focus of farmers and consultants in recent years. In order to make recommendations on how best to support the provision of the ecosystem service ‘bioregulation’ and thus benefit from it in the long-term, knowledge of the key organisms involved and a deeper understanding of the regulation by external factors are needed.
Several studies have been carried out on different groups of organisms, covering various size classes from microorganisms to macrofauna (e.g., Goncharov et al. 2020; Schrader et al. 2013). Concerning soil fauna, the results suggest that in particular the anecic primary decomposers within the earthworm community, which show a food preference for fungal-infected plant material, are suitable antagonists with high bioregulatory potential (Meyer-Wolfarth et al. 2017; Schrader et al. 2013; Wolfarth et al. 2011). In the agroecosystems of temperate regions, the earthworm species *Lumbricus terrestris* is a particularly promising representative, as it occurs in high densities in unploughed arable soils (Briones and Schmidt 2017; van Capelle et al. 2012) and prefers *Fusarium*-infected plant material as a food source (Bonkowski et al. 2000; Goncharov et al. 2020). Furthermore, there are indications that this species also promotes the degradation of *Fusarium* mycotoxins in plant residues (Oldenburg et al. 2008; Schrader et al. 2009; Wolfarth et al. 2016). However, all these studies relate exclusively to wheat and, concerning mycotoxins, to deoxynivalenol (DON). Studies on *L. terrestris* in suppressing *Fusarium* and degrading various *Fusarium* mycotoxins in maize residues are lacking so far. Recommendations to farmers often emphasise the importance of chaff size in suppressing *Fusarium* infections. In this context, the general rule applies: the finer, the better. However, the relevance of size ranges for pathogen suppression and mycotoxin degradation has not yet been investigated.

Against the background of these gaps in knowledge, the objective of the present field study was to analyse the effectiveness of the bioregulatory potential of the earthworm species *L. terrestris* in suppressing the three *Fusarium* species *F. graminearum*, *F. culmorum*, and *F. verticillioides* and in accelerating the degradation of the main mycotoxins DON, 3-acetylatedoxynivalenol (3-AcDON) and zearalenone (ZEN) in the maize mulch layer. Two different size classes of maize remains (fine straw and coarse straw) were considered to detect and assess residue size-specific differences.

The present study aims at testing the following overall hypothesis: earthworms are crucial bioregulators within the dynamic of the ecosystem service/disservice balance in maize residue mulching systems.

### Materials and methods

#### Environmental conditions and site description

The experiment was conducted in the reduced tillage plots of a long-term soil tillage field trial located near Göttingen in Germany (study site: ‘Garte Süd’). Details on geographic location, climate, soil conditions, and set up of the field trial are given in Table 1.

The experiment was carried out in late summer 2018 after the harvest of rape. During the experimental time of six weeks, the mean air temperature was about 13.7 ± 0.6 °C; the total precipitation was 68.1 l m$^{-2}$.

#### Soil

Topsoil (Haplic Luvisol) was collected from the reduced tillage plots of the field trial. The soil was air-dried and stored at 4 °C until further processing. Shortly before the experiment started, the soil was macroscopically cleared of organic residues, sieved using a mesh size of 2 mm, and defaunated by three consecutive freezing (−18 °C) and thawing (room temperature) cycles of 24 h each. Finally, the soil was moistened to 17.34% (w/w), which corresponds to a water holding capacity (WHC) of about 60%.

#### Maize residues

In 2017, silage maize (*Zea mays*, cultivar ‘Werena’) was cultivated at an experimental site of the Julius Kühn Institute in Braunschweig (Germany). Maize plants were artificially infected by *Fusarium* spp. injection in early August at the principal growth stage 6 of flowering and anthesis (BBCH 65) (Meier 2018) to receive *Fusarium*-infected and mycotoxin-contaminated residues for the experiment. Each maize plant was inoculated by injecting 0.5 ml spore suspension directly into the stem between the first and the second node. The spore suspension contained spores of three strains of the species *F. graminearum* in equal proportions. The total spore concentration was 250,000 spores ml$^{-1}$. The preparation of the spore suspension was carried out according to Oldenburg and Ellner (2015), who give a detailed description of the spore suspension preparation.

The maize stalks were chopped and divided into two size classes after harvesting: coarse straw (5–6 cm) and fine straw (1–2 cm). The material was air-dried until further processing. Although the maize plants were...
artificially infected only with the species *F. graminearum*, the maize stalks contained DNA from *F. graminearum* and, additionally, *F. culmorum* and *F. verticillioides* after harvest (Table 2). Initially, the mycotoxins DON and 3-AcDON were found in both maize residue size classes (Table 2).

**Earthworms**

Individuals of the primary decomposing anecic earthworm species *Lumbricus terrestris* were purchased from a commercial provider (Superwurm e.K., Düren, Germany). Two weeks before the start of the experiment, 24 adult individuals (clitellate) were adapted to the soil from the field trial at 17 °C (± 1 °C). During this adaptation period, earthworms were kept in plastic containers and fed with non-infected control maize material. Before individuals were inserted into the experimental units (mesocosms), they were transferred into tap water to remove adherent organic material and mucus. Their biomass was determined by weighing (± 0.01 g).

**Mesocosms as experimental units**

Cylindrical mesh-bags made of nylon-gauze (diameter: 12 cm, height: 30 cm) were used as experimental units (mesocosms). A mesh size of 15 μm ensured an exchange of air, water, and soluble nutrients with the surrounding soil in the field, but prevented other soil fauna from immigrating and earthworms and ascospores from escaping. Shortly before the experiment started, 24 mesocosms were filled with 1500 g (dw) moistened soil. The soil was compacted to a soil column of about 10 cm height, resulting in a bulk density similar to field conditions (Table 1). Two adult *L. terrestris* individuals were put into one half of the mesocosms (earthworm treatment), whereas the other half represented a non-faunal control (control). The weight-based allocation of individuals to mesocosms ensured nearly similar earthworm biomasses per experimental unit and treatment. Mean earthworm biomass per mesocosm was 8.7 ± 0.2 g. When earthworms burrowed completely into the soil, 10 g of artificially infected, air-dried, and chopped maize stubbles were added to each mesocosm’s soil.

**Table 1** Site description and environmental conditions (climate, soil) of the field site ‘Garte Süd’ as well as experimental design of the long-term field trial.

| Site | continental/atlantic |
|---|---|
| Biogeographic region |  |
| Geographic location | 9 km south of Göttingen (DE) |
| GPS coordinates | 51°29′ N, 9°56′ E |
| Altitude | 163 m a.s.l. |
| Climate |  |
| Mean annual air temperature | 9.5 °C |
| Mean annual precipitation | 621 mm y⁻¹ |
| Soil |  |
| Type | Haplic Luvisol |
| Texture | Ut4 (19% clay, 68% silt, 13% sand) |
| pH | 7.7 |
| TOC | 0.94% |
| TN | 0.10% |
| CEC | 11.5 cmol⁺ kg⁻¹ |
| Bulk density | 1.30 Mg m⁻³(a) |
| Long-term field trial |  |
| Start | 1970 |
| Plot size | 20 m x 40 m |
| Tillage | conventional tillage (plough, *n*=8), reduced tillage (rotary harrow, *n*=8) |
| Crop rotation | cereal-legume based (no repeated rotation) (2018: rape) |

(a) Jacobs et al. (2009)
surface (about 113 cm²) and moistened by spraying with tap water (about 3 ml). This amount of plant material is equivalent to 8.8 t ha⁻¹, representing standard mulching conditions for maize residues under reduced tillage (Morel 1996). Half of the mesocosms of each soil fauna treatment (earthworm treatment and control) received maize residues of one size class each (fine straw or coarse straw). Finally, mesocosms were closed by plastic clips.

Experimental design

The experiment was carried out during six weeks from September 5 to October 10. In total, 24 mesocosms were established in the reduced tillage plots of the long-term field trial. The experiment comprised mesocosms of four different treatment combinations with \( n = 6 \) per treatment: control/coarse straw, control/fine straw, earthworm treatment/coarse straw, and earthworm treatment/fine straw. Mesocosms were arranged in blocks of four (one per treatment with a distance of 1 m). One block of four mesocosms was inserted into each of six randomly selected reduced tillage plots, resulting in a replication of six per treatment. Mesocosms were buried in close contact with the surrounding soil, with the soil surface within and around mesocosms being at the same level.

Due to Germany’s dry weather conditions in 2018, mesocosms and surrounding soil were irrigated weekly by adding 10.4 l m⁻² of tap water per mesocosm. This measure was necessary to prevent the earthworms from entering a dormant stage to survive the unfavourable weather conditions.

Determination of soil surface cover

Photographs of each mesocosm’s soil surface area were taken at the beginning and the end of the field experiment. By scanning these top view photographs, relative shares [%] of uncovered soil or casts and areas covered with maize residues were evaluated using a colour analysis software specially developed to determine the degree of soil surface coverage (Programm zur Analyse von Bodenbedeckungsgraden aus Digitalfotos © 2005–2007 by Ulf Böttcher (CAU Kiel, Germany)).

Sampling and sample processing

After six weeks of field exposure, mesocosms were removed from the field plots and taken to the laboratory. Mesocosms were opened, and the straw remaining on the soil surface was removed macroscopically. Maize residues were mechanically cleaned from adhesive soil (to prevent potential leaching of mycotoxins (Gautam and Dill-Macky 2012), washing was avoided), dried at 30 °C and finely ground using a batch mill (A10 basic, IKA®-Werke GmbH & Co.KG, Staufen, Germany). The ground plant material was further used to determine Fusarium species DNA amounts and to analyse mycotoxin concentrations.

Earthworms were removed from the soil columns, transferred to cold tap water to wash off adhering soil and organic material, and weighed individually. Finally, two soil samples were taken from the well-mixed material of each soil column. The samples of one subset were used for the gravimetric determination of soil moisture after 24 h drying at 105 °C. The samples of the second subset were dried at 30 °C and manually ground (< 0.5 mm particle size) using a mortar and pestle. These samples were used for the determination of mycotoxin concentrations.

Determination of Fusarium species DNA amounts

Table 2 Initial Fusarium species DNA amounts (F. graminearum, F. culmorum, F. verticillioides) and mycotoxin concentrations (DON, 3-AcDON, ZEN, NIV, FB₁, and FB₂) ± standard errors [μg kg⁻¹] in both maize residue size classes (coarse straw, fine straw) at the beginning of the field experiment. ND = not detected

| Cultivar               | ‘Werena’         |        |        |
|-----------------------|------------------|--------|--------|
| Residue size class    | Coarse straw     | Fine straw |
| DNA amount [μg kg⁻¹]  |                  |        |        |
| F. graminearum        | 10±2             | 18±9   |
| F. culmorum           | 19,929±6956      | 2685±951 |
| F. verticillioides     | 96,072±39,224    | 110,175±36,290 |
| Mycotoxin concentration [μg kg⁻¹] |        |        |        |
| DON                   | 3717±490         | 6405±650 |
| 3-AcDON               | 4233±352         | 8661±2745 |
| ZEN                   | ND               | ND     |
| NIV                   | ND               | ND     |
| FB₁                   | ND               | ND     |
| FB₂                   | ND               | ND     |
were determined using a quantitative real-time PCR (qPCR) based on TaqMan® technology to quantify the abundances of the three *Fusarium* species. For this purpose, the methods described in Brandfass and Karlovsky (2008) and Hogg et al. (2007) were modified. DNA was extracted from 1.0 g finely ground sample material. The qPCR was performed by an analytical laboratory specialized in the diagnosis of phytopathogens using biomolecular technologies (IDENTXX GmbH, Stuttgart, Germany). Detailed information on the methodological procedure can be obtained from IDENTXX GmbH, Stuttgart.

**Determination of *Fusarium* mycotoxin concentrations**

Initial maize residues as well as maize and soil samples from all mesocosms at the end of the experiment were analysed for the presence of the *Fusarium* mycotoxins DON, 3-AcDON, ZEN, nivalenol (NIV), fumonisin B1 (FB1), and fumonisin B2 (FB2). For the determination of mycotoxin concentrations, 1.0 g finely ground maize residues and 5.0 g homogenised soil material were extracted by turbulent shaking for 30 min. An acetonitrile/water mix (50:50) was used as an extraction solution. The extracts were then diluted 1:10 with 30% methanol. 10 μl of the purified filtres were analysed by a Thermo scientific DIONEX UltiMate 3000 HPLC system. The column was a Phenomenex Kinetex C18 (2.6 μm, 100 mm, 3 mm i.d.). The mobile phase consisted of solvent A (methanol +0.5% acetic acid +5 mmol ammonium acetate) and solvent B (water +0.5% acetic acid +5 mmol ammonium acetate). A gradient procedure was used as followed: starting with 2% of A: up to 98%. The flow rate was 300 μl/min, and the column temperature was set at 40 °C. The HPLC was coupled with the mass spectrometer QTRAP 5500 (AB SCIEX) used in electrospray ionization mode.

The detection limit for DON, 3-AcDON, ZEN, NIV, FB1, and FB2 was 1 μg kg⁻¹. For more information on mycotoxin determination, see Oldenburg and Ellner (2015).

**Statistics**

Changes in *Fusarium* DNA levels, mycotoxin concentrations, and accompanying parameters (soil moisture, soil surface cover, earthworm biomass) during the experimental period were calculated using the formula \(\log(X(t_1)/X(t_0))\), where \(X\) is the respective value of a parameter at the start time \(t_0\) and the end time \(t_1\) of the experiment (Crawley 2007), to ensure relative comparability between increases and decreases. In cases where either initial or final values were zero (ZEN concentration), one was added to both concentrations to determine comparable value shifts. Impacts on the log-rates of change were analysed using linear mixed-effect models. Due to the nested experimental design, plots were generally considered as a random factor.

Changes of all parameters were analysed for an effect of the residue size class. In terms of soil moisture, pathogen suppression, and mycotoxin degradation, moreover, the soil fauna treatment (control vs. earthworm treatment) and the two-way interaction between both factors were integrated into the model.

Since the incorporation of maize residues into the soil presupposes earthworms’ presence and activity, the statistical evaluation of the decrease in soil surface cover refers exclusively to the mesocosms with earthworms.

Model residuals were checked visually (normal quantile-quantile plots (QQ plots), residual plots, boxplots) and by testing procedures (Shapiro-Wilk normality test, Levene test) to evaluate normality and homogeneity of variance. In cases in which the assumption of the normal distribution of residuals or the homogeneity of variances was violated, data were transformed by either exponential transformation (\(e^x\)) (data sets: *F. graminearum* DNA amount, 3-AcDON and ZEN concentrations) or square root transformation (data set: earthworm biomass) to fulfill the model requirements.

Analysis of Deviance (Type II Wald chi-square test) was performed to analyse the impacts of explanatory variables and their interaction. Correlations between parameters were analysed by use of Pearson correlation.

To make comparative statements on the relevance and regulation of individual species and mycotoxins, changes of relative proportions [%] within the overall spectrum were analysed. To investigate whether percentages of the three *Fusarium* species and the different mycotoxins in maize residues changed under field conditions and depending on treatment, a PERMANOVA was performed (number of permutations: 9999). Two different comparisons were carried out for each of the two residue size classes: firstly, a comparison of the initial material with the control material after completion of the experiment, and secondly, a comparison between the control material and the material from the earthworm treatment at the end of the experimental period. In terms
of the first comparison, time (start vs. end) and residue size class; in terms of the second comparison, fauna treatment, residue size class, and plot were considered as explanatory variables.

All statistics were performed using R version 3.5.0 (R Core Team 2018). The packages lme4 (Bates et al. 2015), MASS (Venables and Ripley 2002), and car (Fox and Weisberg 2019) were used for linear mixed modelling. The package corplot (Wei and Simko 2017) was used to analyse Pearson correlations, the package vegan (Oksanen et al. 2019) for analysis of relative changes of DNA amounts and mycotoxins via PERMANOVA. The figure was created using the ggplot2 package (Wickham 2016).

Results

Soil moisture

In the course of the experiment, the soil moisture in the mesocosms decreased by about 9.3 ± 0.6%, resulting in an average water holding capacity of 58.56 ± 0.36% at the end of the experimental period (Supplementary Table S1). Soil moisture changes differed significantly between control (−10.8 ± 0.7%) and earthworm treatment (−7.8 ± 0.7%) (Table 3), resulting in higher average soil moisture in mesocosms with earthworms compared to the control (Supplementary Table S1). No significant residue size class or interaction effects were detected (Table 3).

Soil surface cover

During the experimental period, earthworms incorporated plant material into the soil. Accordingly, in mesocosms with *L. terrestris*, the surface area of soil covered by maize residues decreased, whereas it remained unchanged at 100% in the control (Supplementary Table S1). Since fine straw was incorporated into the soil more effectively than coarse straw, the decrease of soil surface cover significantly differed between residue size classes (fine straw: −16.0 ± 1.8%, coarse straw: −5.1 ± 1.3%) (Supplementary Table S1, Table 3). No correlation between changes in soil moisture and soil surface cover was detected (Supplementary Table S2).

Fusarium DNA amounts

DNA amounts of all three *Fusarium* species in maize residues increased during the duration of the experiment, independent of treatment (Fig. 1, Supplementary Table S3). Average rates of increase ranged from eight (*F. verticillioides* in the coarse straw of the control) to 17,200 (*F. culmorum* in the fine straw of the control) times the initial value. Average increases in amounts of *F. graminearum* DNA were significantly higher in the control (200-fold) compared to the earthworm treatment (about 40-fold) and in fine straw (approximately 200-fold) times the initial value.

Recapture rate and biomass of earthworms

After completion of the experiment, the loss of one of a total of 24 *L. terrestris* individuals was recorded. The recapture rate was thus 96%. All recaptured individuals were active. None of them entered a dormant stage.

Mean earthworm biomass decreased during the experimental period by about −7.3 ± 1.4% (Supplementary Table S1). Reduction rates did not significantly differ depending on residue size class (Table 3) and were not correlated with changes in soil moisture or soil surface cover (Supplementary Table S2).

Table 3  Chisquare ($\chi^2$) values and significance levels of Analysis of Deviance (Type II Wald Chisquare Test) on the effects of fauna treatment (control vs. earthworm treatment) and residue size (coarse straw vs. fine straw) on changes of soil moisture, soil surface cover and earthworm biomass [%]. Significant results are printed in bold. Degrees of freedom (Df) for all terms equal 1. NA = not applicable/no data available.

|                      | Soil moisture | Soil surface cover | Earthworm biomass |
|----------------------|---------------|--------------------|-------------------|
| Fauna treatment (F)  | 9.017**       | NA                 | NA                |
| Residue size (R)     | 0.255         | 21.083***          | 1.252             |
| F x R                | 0.404         | NA                 | NA                |

Significance codes: < 0.001***; < 0.01**; < 0.05*; < 0.1 ( *)
compared to coarse straw (about 50-fold) (Fig. 1, Table 4). The earthworm-induced suppression of fungal growth, quantified as smaller increases in \( F. graminearum \) DNA levels compared with the control, tended to be more pronounced in fine straw than in coarse straw (Fig. 1, Table 4). Average increases in \( F. culmorum \) DNA amounts were significantly higher in fine straw (about 15,000-fold) than in coarse straw (about 1000-fold), but showed no differences depending on the presence of earthworms (Fig. 1, Table 4). DNA amounts of \( F. verticillioides \), by contrast, showed a tendency of a stronger increase in the earthworm treatment (about 20-fold) compared to the control (10-fold) but did not differ depending on residue size class (Fig. 1, Table 4).

The shifts in DNA quantities of \( F. culmorum \) and \( F. verticillioides \) were positively correlated \((r = 0.473, p = 0.020)\) (Supplementary Table S2). In the earthworm treatment, \( F. culmorum \) DNA amounts increased with decreasing soil surface cover \((r = -0.595, p = 0.041)\) (Supplementary Table S2), indicating increased growth rates of this species after incorporation of maize residues into the soil.

**Mycotoxin concentrations**

At the end of the experiment, the three \( Fusarium \) mycotoxins DON, 3-AcDON, and ZEN were detected in the maize residues (Fig. 1, Supplementary Table S4). FB1 was found in low concentration in only one sample (coarse straw in the control) (Supplementary Table S4). Contamination of maize residues with the mycotoxins NIV and FB2 has not been detected at any time. While DON and 3-AcDON were already present in the initial material, ZEN was newly formed during the field experiment. Average concentrations of DON and 3-AcDON decreased during field exposure. Rates of decrease were significantly higher in the earthworm treatment (DON: \(-70.0 \pm 2.5\%\), 3-AcDON: \(-97.7 \pm 0.5\%)\) compared to the control (DON: \(-33.0 \pm 11.6\%,\) 3-AcDON: \(-92.4 \pm 1.6\%)\) (Fig. 1, Table 4). Analogously, average rates of new ZEN formation were also significantly lower in mesocosms with earthworms.

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**Fig. 1** Changes in DNA amounts (DNA) of the species \( F. graminearum, F. culmorum, \) and \( F. verticillioides \) (shown above) and in concentrations (conc.) of the mycotoxins DON, 3-AcDON and ZEN (shown below) \( \pm \) SE [\( \mu g \) kg\(^{-1}\)] during the experimental runtime, presented as \( \log(X(t_{1})/X(t_{0})) \), with \( t_{0} = \) start time and \( t_{1} = \) end time of the experiment, in the control (C) and the earthworm treatment (E), shown for both residue size classes (coarse straw, fine straw). Stars indicate arithmetic means. Values <0 indicate a decrease, values >0 an increase.
compared to the control (Fig. 1, Table 4). An effect of residue size class, with a significantly higher decrease in fine straw (−62.6 ± 5.6%) than in coarse straw (−40.3 ± 12.1%), was only detected for DON (Fig. 1, Table 4). No significant effect of the interaction between earthworm treatment and residue size class was detected for any of the three toxins (Table 4).

Changes in 3-AcDON contamination were positively correlated with shifts in both, DON (r = 0.580, p = 0.003) and ZEN (r = 0.424, p = 0.039) concentrations (Supplementary Table S2).

No mycotoxins could be detected in the soil samples, as concentrations were generally below the detection limit.

The results of the linear mixed-effect models on the effects of soil fauna treatment (control vs. earthworm treatment), maize residue size (coarse straw vs. fine straw), and the interaction between them on the (partially transformed) log-rates of changes in accompanying parameters (soil moisture, soil surface cover and earthworm biomass), Fusarium species DNA amounts, and mycotoxin concentrations are given in the supplementary Tables S5 and S6.

Proportional analysis

In the initial maize residues applied to the mesocosms, F. verticillioides represented the dominant Fusarium species in both residue size classes with mean proportions of about 71% in coarse straw and about 96% in fine straw (Table 5). Accordingly, the relative proportion of F. culmorum was higher in coarse straw (about 29%) than in fine straw (about 4%). Less than 0.1% of the Fusaria present in both residue size classes belonged to F. graminearum (Table 5).

During the experiment, this distribution shifted significantly (Table 6) in favour of F. culmorum, which accounted for more than 80% in the control at the end of the experiment (Table 5). This shift was significantly higher in fine straw than in coarse straw (Tables 5 and 6). The comparison of the Fusarium community in maize residues from the control with that in residues from the earthworm treatment affirms a tendency towards higher proportions of F. culmorum and lower percentages of F. verticillioides in fine straw compared to coarse straw (Tables 5 and 6). The presence of earthworms did not significantly affect the shifts in species’ relative abundances (Table 6).

Regardless of the residue size class, about 54% of the mycotoxin contamination in the initial material was 3-AcDON and about 46% DON. ZEN and FB1 were not detected (Table 5). In the course of the experiment, this ratio shifted significantly (Table 6), resulting in a final contamination in which DON accounted for the highest toxin content at over 76% (Table 5). About 10% of the toxin load was 3-AcDON and between 11 and 14% the species in both residue size classes with mean proportions of about 71% in coarse straw and about 96% in fine straw (Table 5). Accordingly, the relative proportion of F. culmorum was higher in coarse straw (about 29%) than in fine straw (about 4%). Less than 0.1% of the Fusaria present in both residue size classes belonged to F. graminearum (Table 5).

### Table 4

| Fusarium DNA amount | Mycotoxin concentration |
|---------------------|-------------------------|
| **F. graminearum**  | **DON**                 |
| Fauna treatment (F) | 10.529***               |
| Residue size (R)    | **6.283***               |
| F x R               | 3.395(+)                |
| **F. culmorum**     |                         |
| Fauna treatment (F) | 1.262                   |
| Residue size (R)    | **48.243***              |
| F x R               | 0.300                   |
| **F. verticillioides** | **3-AcDON**         |
| Fauna treatment (F) | 3.096(+)                |
| Residue size (R)    | 2.369                   |
| F x R               | 0.333                   |

Significance codes: < 0.001***; < 0.01**; < 0.05*; < 0.1(*)
newly formed ZEN (Table 5). These shifts within the toxin spectrum were independent of the residue size (Table 6). Relative proportions of Fusarium toxins did not differ significantly between maize residues from the control and those from the earthworm treatment (Table 6).

Discussion

In the present study, the ecosystem service/disservice balance provides information on the relationship between infestation pressure as well as mycotoxin contamination by phytopathogens (here: three Fusarium species) and the bioregulatory potential of natural antagonists (here: L. terrestris). The regulatory processes observed in the control treatment exclusively indicate soil microbial activity, while those in the earthworm treatment reflect single and interaction effects between earthworms (incl. associated microorganisms) and soil microorganisms. The findings help to better understand natural bottom-up bioregulation pathways in maize cultivation and to evaluate their effectiveness in the context of the synergy effect between farmer and soil fauna (Meyer-Wolfarth et al. 2017; Schrader et al. 2020). The knowledge gained about the functional relationships and interactions is of great relevance both now and in the future, as reduced tillage in combination with mulching techniques is becoming increasingly important worldwide as a contribution to sustainable agricultural production, including in maize cultivation (Claassen et al. 2018; Kassam et al. 2009).

### Table 5

| Fusarium DNA amount [%±SE] | Fusarium mycotoxins [%±SE] |
|---------------------------|---------------------------|
| **F. graminearum** | **F. culmorum** | **F. verticillioides** | DON | 3-AcDON | ZEN | FB_1 |
| Coarse straw | Start | 0.07±0.05 | 29.06±10.05 | **70.88±10.10** | 46.41±1.31 | **53.59±1.31** | ND | ND |
| Con. | 0.01±0.00 | 82.60±6.29 | 17.38±6.29 | **76.04±5.21** | 9.64±2.79 | 14.23±5.23 | 0.09±0.08<sup>(a)</sup> | |
| Earthw. | 0.01±0.00 | 84.60±6.03 | 15.39±6.03 | **80.73±5.28** | 5.78±1.95 | 13.49±4.90 | ND | |
| Fine straw | Start | 0.02±0.01 | 4.03±1.97 | **95.95±1.97** | 45.69±4.74 | **54.31±4.74** | ND | ND |
| Con. | 0.02±0.01 | 94.72±1.04 | 5.26±1.04 | **78.96±3.41** | 10.37±1.62 | 10.67±3.95 | ND | |
| Earthw. | 0.01±0.00 | 92.08±0.36 | 7.91±0.36 | **85.75±2.88** | 8.60±2.67 | 5.66±1.58 | ND | |

<sup>(a)</sup> detected in one sample

### Table 6

| Sums of Sqs. | R² | p value |
|--------------|----|---------|
| Initial residues vs. Control |
| DNA amount [%] | Time (T) | 2.0801 | 0.8455 | **0.0002*** |
| | Residue size (R) | 0.0002 | 0.0001 | 0.9155 |
| | T x R | 0.1383 | 0.0562 | **0.0158*** |
| Mycotoxins [%] | Time (T) | 0.7187 | 0.7648 | **0.0001*** |
| | Residue size (R) | 0.0007 | 0.0008 | 0.9431 |
| | T x R | 0.0002 | 0.0003 | 0.9741 |
| Control vs. Earthworm treatment |
| DNA amount [%] | Fauna treatment (F) | 0.0001 | 0.0002 | 0.9345 |
| | Residue size (R) | 0.0577 | 0.1703 | **0.0505*** |
| | Plot | 0.0556 | 0.1642 | 0.6198 |
| | F x R | 0.0032 | 0.0096 | 0.5915 |
| Mycotoxins [%] | Fauna treatment (F) | 0.0177 | 0.0454 | 0.3321 |
| | Residue size (R) | 0.0160 | 0.0410 | 0.3570 |
| | Plot | 0.1084 | 0.2774 | 0.2726 |
| | F x R | 0.0020 | 0.0052 | 0.8690 |

Significance codes: < 0.001***; < 0.01**; < 0.05*; < 0.1<sup>(+)</sup>
The maize straw showed an apparent infestation with *F. culmorum* and *F. verticillioides* at the beginning of the experiment, although only the species *F. graminearum* was artificially injected. Therefore, this infestation is due to natural infection in the field during the growth phase of the maize plants. The high proportion of *F. verticillioides* DNA (over 70%) reveals that this species, which is originally native to warmer and drier regions (Aguín et al. 2014; Bottalico 1998), is already present in temperate latitudes. This result is in line with Czembor et al. (2015) and Pfördt et al. (2020), who have recently detected this species in Poland and Germany. It underlines the assumption of Oldenburg et al. (2018) that *F. verticillioides* will play an increasing role in maize cultivation in temperate latitudes in the future. The relative proportion of the initially inoculated species *F. graminearum*, by contrast, was the lowest within the *Fusarium* community (< 0.1%) at any time and in any treatment. This result supports the assumption of Leplat et al. (2013) and Pereyra and Dill-Macky (2008), who classified the species *F. graminearum* as a relatively weak competitor compared to other *Fusarium* species and within the soil fungal community. The frequently described high competitiveness of this species (Velluti et al. 2000; Xu et al. 2007) is not confirmed in the present study. It is known that several anthropogenic factors, including preceding crops, tillage system, and weed management, can alter the development of the soil biota, which in turn can change the saprotrophic development of *F. graminearum* (Leplat et al. 2013). Thus, differences in farming practice might explain differences in competitive ability besides the origin of fungal species and isolates (see below).

During the mesocosm experiment, DNA levels of all three *Fusarium* species in maize residues increased in the control treatment. The main reason for this biomass increase was probably the enhanced water availability under field conditions compared to the initial dried material, which represents the most essential factor for *Fusarium* growth besides temperature (Belizán et al. 2019). With an arithmetic mean of 14 °C, the temperature during the experimental runtime was well below the respective temperature optimum, but with a value of over 10 °C still in a range in which growth of all three species could be expected (Brennan et al. 2003; Cook and Christensen 1976). The third factor that significantly influences the growth of *Fusarium* species as a function of humidity and temperature is the origin of species and isolates and the time available for their adaptation to specific climatic conditions (temperature ecotypes) (Brennan et al. 2003; Hudec and Muchova 2010; Pettitt et al. 1996). These different adaptation stages to the conditions of temperate latitudes are reflected in the detected growth rates, which were highest for *F. culmorum*, followed by *F. graminearum* and *F. verticillioides* (Fig. 1).

The species *F. culmorum* was the predominant *Fusarium* species in Central and Northern Europe until the 1990s as the main pathogen of maize stem rot (Bottalico 1998). Long-term adaptation to climate conditions in this part of Europe probably contributed to the high biomass increase of *F. culmorum*, which represented the dominant species at the end of the experiment with a share of over 80% in all treatments. The species *F. graminearum*, by contrast, was originally native to warmer and humid regions (Brennan et al. 2003) and has only been present in the colder regions of temperate latitudes since the 1980s (van der Lee et al. 2015; Waalwijk et al. 2003). Currently, *F. graminearum* is often considered the dominant species in most cereal growing areas worldwide (Goswami and Kistler 2004; Mansretta and Rossi 2016). It is frequently described as displacing *F. culmorum* (van der Lee et al. 2015; Waalwijk et al. 2003). Slower growth rates and lower biomasses compared to *F. culmorum*, as demonstrated in the present study, were also detected in wheat by Brennan et al. (2003) and Xu et al. (2007). The third species, *F. verticillioides*, originates from even warmer regions (Aguín et al. 2014). This species probably does not yet show a pronounced adaptation to the variable climatic conditions in late summer or autumn. Thus, its growth rates were the lowest compared to the other two *Fusarium* species in the present study. Overall, the demonstrated growth rates and relative abundances of the three species link the conclusions of Hudec and Muchova (2010) and Pfördt et al. (2020) as they indicate that species and factors of their original latitude are the key factors that determine *Fusarium* growth rates and species spectrum at a specific temperature and humidity in maize cultivation.

Regarding an effect of the maize residue size classes, the two species *F. graminearum* and *F. culmorum* showed a higher DNA increase in fine straw than in coarse straw. This effect can be explained by the fact that the finely chopped material offers a higher proportion of surfaces. This favours the direct contact of *Fusarium* fungi, which are mainly contained in the
maize stalk pith (Zhang et al. 2016), with soil water and nutrients. As the moisture requirements of F. culmorum and F. graminearum are higher compared with the drought-adapted species F. verticillioides (Marín et al. 1996; Torres et al. 2003), they benefited more from the splitting of the plant material. In addition, a finer crushing of the maize straw allows quick colonization by soil-borne fungi and bacteria. Diverse interactions with these soil microorganisms regulate the growth of Fusarium fungi through direct, indirect, and mixed-path mechanisms (Wachowska et al. 2017). Whereas in the literature, mainly antagonistic effects of the soil microbiome against Fusarium species are described (Wachowska et al. 2017), the present results indicate a species-specific increase. In total, these results suggest that, without soil fauna, a smaller chaff size of maize mulch may stimulate specific Fusarium species and even increase the infestation pressure caused by them.

The bioregulatory capacity of the detritivore earthworm species L. terrestris on Fusaria was species-specific: F. graminearum was suppressed, F. culmorum was not affected, and F. verticillioides was slightly promoted. Accordingly, with regard to Fusarium regulation, the hypothesis of the present study was confirmed for only two out of three species, a shift of the service/disservice balance towards service (pathogen suppression) even for only one species. The present results for maize residues contradict the results for wheat straw of Meyer-Wolfarth et al. (2017), Oldenburg et al. (2008), Schrader et al. (2009), and Wolfarth et al. (2011), who demonstrated suppression of F. culmorum by L. terrestris. These different effects suggest that the cultivated plant and potentially even the respective cultivar plays an important role not only for the composition of the Fusarium community (Czembor et al. 2015) and their respective growth rates (Brennan et al. 2003) but also for the interaction between Fusarium species and soil fauna.

The chaff size of maize residues played only a minor role during earthworm-induced regulation of Fusarium growth. The tendency of a stronger suppression in fine straw than in coarse straw was detected for F. graminearum only. Since the earthworm biomass did not differ significantly depending on the residue size class, and the mean weight reduction of less than 10% over six weeks indicates an adequate nutrient supply (Fründ et al. 2010), good usability of both chaff sizes as a food source for L. terrestris can be assumed. The decreasing soil surface cover in the earthworm treatment (fine straw > coarse straw) indicates incorporation of the residues into the soil and the burrow system. According to the ‘external rumen’ principle, which is based on the definition of Swift et al. (1979) and specified for earthworms by Vavelle (1988) and Brown et al. (2000), this organic material is stored in the burrows until it has been further split and pre-decomposed by the microbial community. The stronger suppression of F. graminearum in fine straw was probably not caused by direct feeding of earthworms, but rather by the priming effect of earthworm mucus, being highly bioavailable for soil microorganisms (Binet et al. 1998; Schrader et al. 2013). It can be assumed that due to this effect and the higher soil moisture in the earthworm treatment, competing or antagonistic soil microorganisms colonized the fine straw faster than the coarse straw. Comparable effects regarding a stronger reduction of F. graminearum in strongly split compared to intact maize residues were also demonstrated in field experiments of Vogelsang et al. (2011).

During the experimental period, DON and 3-AcDON were reduced in the control due to microbial degradation and transformation (Vanhoutte et al. 2016; Venkatesh and Keller 2019; Wachowska et al. 2017), which was faster for 3-AcDON compared to DON. While DON and 3-AcDON were already formed in the growing maize plant, ZEN was only produced in the chaff of the maize mulch layer. Unlike DON (Proctor et al. 1995; Snijders 1995), ZEN does not play a significant role as a virulence factor and for disease development in the living plant (Munkvold 2017), but can inhibit the formation of certain soil-borne microorganisms (Bacon et al. 2017) and thereby lead to a competitive advantage in the saprotrophic phase of Fusaria. Thus, this result supports the theory described by Müller et al. (2014) and Venkatesh and Keller (2019) of the formation of specific mycotoxins due to interactions with microorganisms and proves that the toxin composition in the living plant can differ considerably from that in the mulch layer. FB₁ is the most common mycotoxin produced by F. verticillioides in maize (Bottalico 1998; Czembor et al. 2015) and probably plays a role in suppressing the plant’s defense reaction (Galeana-Sánchez et al. 2017). However, in the present study, this mycotoxin was not formed during plant growth and was only detected in a single sample in the mulch layer. In accordance with the results of Ryu and Bullerman (1999), a correlation between growth rates of the three Fusarium
species and detected changes in toxin concentrations was not found in the present study.

Concerning the residue size classes, in line with the study of Vogelgsang et al. (2011), the present results reflect a significantly stronger reduction of DON in fine than in coarse straw, but no effect on the degradation of 3-AcDON or ZEN. Since mycotoxins serve as communication signals in fungal-bacterial interactions, some bacteria possess the ability to degrade or transform certain toxins or either promote or inhibit their formation (Vanhoutte et al. 2016; Venkatesh and Keller 2019). The results of the control treatment reflect that the degradation of Fusarium toxins in the mulch layer specifically depends on the composition of the respective microbial community.

The earthworms (L. terrestris) significantly accelerated the reduction of all three toxins, revealing their bioregulatory potential for mycotoxin degradation. With regard to mycotoxins, the hypothesis of the present study was confirmed and an earthworm-induced shift of the service/disservice balance towards service was demonstrated. At the end of the experiment, the maize material in the control, which was only affected by soil microorganisms, still had 1.8 times the maximum legal level of DON and 1.5 times the maximum level of ZEN for unprocessed maize (EC (European Commission) 2007). By contrast, in the earthworm treatment, maximum legal levels for unprocessed maize were significantly undercut with a 0.8-fold concentration for DON and a 0.5-fold concentration for ZEN. Overall, the earthworms reduced the DON concentration by half and 3-AcDON and ZEN concentrations by two thirds over the experimental period compared to the control. These results are consistent with the studies of Meyer-Wolfarth et al. (2017), Oldenburg et al. (2008), Schrader et al. (2009), and Wolfarth et al. (2011), who demonstrated a reduction of DON in wheat straw by L. terrestris. Effects of earthworms thereby resulted from a combination of direct and indirect bioregulatory processes. The feeding activity reduced the mycotoxin concentration by degradation processes in the course of intestinal passage, presumably with the participation of the intestinal flora (Schrader et al. 2013). For DON, this is shown by the studies of Oldenburg et al. (2008) and Schrader et al. (2009), in which it was demonstrated that DON concentrations in the intestine of L. terrestris were significantly lower than in wheat straw and even below the detection limit in casts (Wolfarth et al. 2011). Beyond that, earthworms secrete mucus and coelomic fluid through dorsal pores in their body wall, produce casts, and create middens. Each of these earthworm products contains highly bioavailable substances that increase microbial activity (Brown 1995) and potentially promote microbial mycotoxin degradation. The promotion of these soil animals, for example through reduced or no-tillage where soil conditions permit, the reduction of soil compaction to a necessary level, and the demand-oriented application of agrochemicals, can hence make a significant contribution to reducing mycotoxin contamination of crop residues.

Conclusion

In maize cultivation, the earthworm species L. terrestris represents a key species within the soil fauna community for a species-specific bioregulation of Fusarium species. L. terrestris can shift the ecosystem service/disservice balance in arable systems in both directions, depending on Fusarium species involved in the infestation. Since L. terrestris does not generally contribute to suppressing all Fusarium species relevant in maize cultivation, management decisions have to be made site-specifically and depending on the respective infestation. However, in this context, it should be taken into account that ploughing, as an often-recommended preventive measure, is not an all-round solution. Although it has been proven that incorporation of crop residues into the soil can reduce the frequency of F. culmorum and F. graminearum, it increases that of F. verticillioides (Pfordt et al. 2020).

The potential of L. terrestris to reduce mycotoxin concentrations seems to be independent of the crop and includes DON, 3-AcDON, and ZEN. Agricultural management that considers the needs and habitat requirements of earthworms can thus lead to a synergy in which the interaction of anthropogenic top-down effects (agricultural management) and natural bottom-up effects (bioregulation by earthworms) contributes to sustainable agricultural production on healthy and fertile soils. By accelerating toxin degradation in the mulch layer, L. terrestris has the potential to shift the ecosystem service/disservice balance towards services (toxin degradation) in reduced tillage systems and to keep soils healthy and productive in the long run.
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Code availability Not applicable.

Declarations

Conflict of interest The authors have no conflicts of interest to declare that are relevant to the content of this article.

Consent to participate Not applicable

Consent for publication Not applicable.

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